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RECOMBINANT DNA RESEARCH
Volume 10

Documents Relating to "NIH Guidelines for Research Involving Recombinant DNA Molecules" March 1985 - December 1985

May 1986

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service National Institutes of Health

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Documents Relating to
"NIH Guidelines for Research
Involving Recombinant DNA Molecules"
March 1985 - December 1985

May 1986

Prepared by the
Office of Recombinant
DNA Activities

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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PREFACE

This document is the tenth in a series constituting a "public record" of activities in regard to the National Institutes of Health (NIH) Guidelines for recombinant DNA research.

The first nine volumes cover events from February 1975 through March 1985. This tenth volume covers events from March 1985 through December 1985.

Volumes 1 through 5 in this series may be purchased from the Superintendent of Documents, U.S. Government Printing Office (GPO), Washington, D.C. 20402, or viewed in some 600 public libraries of the GPO depository system. The GPO stock number of Volume 1 is 017-004-00398-6; Volume 2, 017-040-00422-2 and (supplement: Environmental Impact Statement) 017-040-00413-3; Volume 3, 017-040-00429-0 and (appendices) 017-040-00430-3; Volume 4, 017-040-00443-5 and (appendices) 017-040-00442-7; and Volume 5, 017-040-00470-2. Volumes 6, 7, 8 and 9 are available from the Office of Recombinant DNA Activities, National Institutes of Health, Building 31, Room 3B10, Bethesda, Maryland 20892.

CONTENTS

Minutes of the Recombinant DNA Advisory Committee (RAC) Subworking Group on Human Gene Therapy meeting, March 15, 1985 1

Federal Register of March 28, 1985, announcing proposed actions to be considered at a meeting of the RAC, May 3, 1985. 81

Minutes of the RAC Working Group on Human Gene Therapy meeting, April 1, 1985 86

Federal Register of April 15, 1985 announcing the availability for public comment of the "Environmental Assessment and Finding of No Significant Impact" concerning the application to field test ice-nucleation-minus bacteria 116

Minutes of the RAC meeting, May 3, 1985. 120

Memorandum of June 19, 1985, analyzing public comment on, and containing the "Environmental Assessment and Finding of No Significant Impact" concerning the application to field test ice-nucleation-minus bacteria. 165

Federal Register of July 23, 1985, announcing the availability of analysis of public comments on the "Environmental Assessment and Finding of No Significant Impact". 304

Minutes of the RAC Working Group on Toxins meeting, August 16, 1985. . 305

Federal Register of August 19, 1985, announcing proposed actions to be considered at a meeting of the RAC, September 23, 1985 323

Minutes of the RAC Subworking Group on Human Gene Therapy meeting, September 20, 1985. 330

Minutes of the RAC meeting, September 23, 1985 382

Minutes of the RAC Working Group on Viruses meeting, November 12, 1985. 440

Federal Register of November 13, 1985, promulgating an action involving field testing of plants grown from seeds containing recombinant DNA and an Environmental Assessment and Finding of No Significant Impact. 468

Federal Register of November 22, 1985, promulgating actions recommended at the RAC meeting, September 23, 1985 525

Minutes of the RAC Working Group on Gram Positive Bacteria meeting, December 2, 1985. 532

Minutes of the RAC Working Group on Human Gene Therapy meeting, December 16, 1985. 567

Selected letter and document in the period March 1985 to December 1985 relevant to the NIH Guidelines 592

Index of letters and documents 640

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE
SUBWORKING GROUP ON HUMAN GENE THERAPY
MINUTES OF MEETING[1]

MARCH 15, 1985

The Subworking Group on Human Gene Therapy of the Recombinant DNA Advisory Committee was convened at 9:00 a.m. on March 15, 1985, at the National Institutes of Health, Building 31, Room 5A16, 9000 Rockville Pike, Bethesda, Maryland 20205. The meeting was open to the public. Dr. LeRoy Walters was the Chair. The following people were present for all or part of the meeting:

Subworking Group Members:

W. French Anderson
Judith Areen
James Childress
Susan Gottesman
Robert Murray
LeRoy Walters
William J. Gartland
(Executive Secretary)

A full working group roster is attached. (Attachment I)

Other National Institutes of Health Staff:

Elizabeth Milewski, NIAID

Liaison Representative:

Henry Miller, Food and Drug Administration

[1]The subworking group is advisory to the RAC, and its recommedations should not be considered as final or accepted.

Dr. Walters called the meeting of a Subworking Group on Human Gene Therapy to order at 9:10 a.m. on March 15, 1985.

Dr. Walters expressed hope that the subworking group could identify the major issues in the comments received in response to the January 22, 1985, Federal Register (50 CFR 2940) announcement of the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" (Attachment II). The subworking group would discuss appropriate responses to the comments.

Ms. Areen asked whether the working group would explain its responses, particularly responses to comments not incorporated into the points to consider document. She asked whether the subworking group would attempt to draft these explanations at the March 15, 1985, meeting.

Dr. Walters felt justifications for working group decisions vis-a-vis the comments would be necessary and should be publicly available. He did not feel, however, that the subworking group should attempt to draft justifications at the March 15, 1985, meeting.

Dr. Anderson asked whether the working group would continue to accept comments after the close of the official public comment period. He felt the working group should accept all comments offered on the points to consider document. The subworking group agreed; they did not feel, however, that justifications need be offered on comments received after the close of the official comment period.

Dr. Gottesman asked if the document developed by the working group in response to the comments would be republished in the Federal Register prior to the next Recombinant DNA Advisory Committtee (RAC) meeting. Dr. Gartland said the publication deadline for the Federal Register announcing the next RAC meeting would be April 20, 1985. Since the full working group must also consider the comments, it would be difficult to revise the points to consider document in the time available. He noted that other working groups in the past have simply published points to consider documents in the Recombinant DNA Technical Bulletin and notified the Institutional Biosafety Committees (IBCs).

Dr. Walters asked how many copies of the January 22, 1985, Federal Register had been mailed to interested individuals. Dr. Gartland replied that several thousand copies had been mailed. Included in this number were individuals who had signed a petition sponsored by the Foundation on Economic Trends.

Dr. Walters called the attention of the subworking group to a handout (Attachment III) which collates the comments (Attachment IV) received in response to the January 22, 1985, Federal Register. He began by noting the comment offered by Dr. Henry Miller of the Food and Drug Administration (FDA) on the introduction to the points to consider document. Dr. Miller had suggested the phrase "and FDA regulations (21 CFR, Parts 50 and 56)" should be added to the introduction following the reference to the Department of Health and Human Services (DHHS) human subject regulations (45 CFR 46). The subsequent sentence should be modified to indicate that the words "these regulations" refer to the DHHS regulations.

Ms. Areen did not agree with Dr. Miller's suggestion to include a reference to FDA's human subject regulations. She questioned whether the Institutional Review Boards (IRBs) followed these FDA regulations. Dr. Murray said IRBs review proposals under their specific guidelines while the FDA reviews proposals under its statutes. He did not understand the purpose of Dr. Miller's suggestion.

Dr. Gartland questioned whether the points to consider document should attempt to define the boundaries of the National Institutes of Health (NIH) and FDA review. The subworking group agreed it would not attempt to define boundaries.

Dr. Gottesman thought the introduction of the points to consider document might simply inform investigators that FDA has human subject regulations. Dr. Anderson suggested modifying to this end the sentence indicating RAC consideration may proceed simultaneously with other agencies' reviews.

Dr. Walters thought the subworking group might check with the NIH Office for Protection against Research Risks (OPRR) to determine whether FDA human subject regulations should be mentioned in this section of the document.

Dr. Walters then called the attention of the subworking group to Dr. Miller's second comment on the introduction to the points to consider document. Dr. Miller had suggested the language dealing with proprietary information should note that the option to hold closed sessions will be available.

Dr. Walters said companies may protect confidential business information in two ways: (1) by obtaining patents; or (2) through trade secrets. Information is protected in the U.S. when the firm files for a patent; the data may then be publicized in open meetings or published. He preferred that submitters of human gene therapy protocols protect business information in this way rather than requesting closed review sessions.

Dr. Gartland said companies may ignore NIH if no provision for closed sessions exists since companies are not required by statute to abide by the NIH Guidelines.

Dr. Anderson said he had no problem with the points to consider document indicating a limited amount of information might be reviewed in closed session.

Dr. Murray felt controversy might arise if the document specifically indicates closed sessions would be held. The current system adequately handles the issue; under that system submitters may request that their proposal be reviewed in closed session.

Dr. Childress wondered whether the introduction should indicate the working group's position on this issue. Dr. Walters suggested the points to consider document might state the working group preference and encourage submitters to apply in a way that permits review in open session. Dr. Gottesman agreed; she said the working group should not commit itself to closed sessions but should not rule out the possibility entirely. She felt the working group could negotiate with submitters as to the amount of material reviewed in closed session even though the working group hopes the initial proposals will not contain proprietary information and may be reviewed in public.

Dr. Anderson did not feel there would be many, if any, initial proposals which would contain confidential information. Dr. Walters said Genentech, Inc., the only firm known at this time to be involved in research involving human gene therapy, does not have a problem with the concept of open session review.

Dr. Childress thought the language in the introduction dealing with open session review was confusing as written; the language both attempts to state working group policy regarding open session review and to encourage open sessions. He suggested this section be redrafted.

Dr. Walters called the attention of the subworking group to Dr. Miller's suggestion to substitute the word "composition" for the word "structure" in Section I-B-1-b.

Dr. Anderson said "structure" and "composition" are different concepts. "Composition" in the chemical sense is a subclass of structure. He thought the word "composition" while it did not contribute a great deal to the data request might, however, be added to this section which would then read: "What are the structure and composition of the material that will be administered to the patient?"

Dr. Anderson suggested Section I-B-1-b-(1) could also be modified to read: "Describe the preparation, structure, and composition of all materials that will be given to the patient or used to treat the patient's cell."

Dr. Anderson suggested the words "nucleic acid" be substituted for the acronym "DNA" in Section I-B-1-b-(1)-(b) of the document.

Dr. Walters said Dr. Miller had suggested two new sections be added to Section I-B-1-b. One section would request the investigator to "describe in detail the methods for harvesting, extraction, and purification, and for the removal of any toxic chemicals introduced by these procedures." The second new section would contain a warning that penicillin and other beta-lactam-containing antibiotics should not be used in the production of materials administered to patients.

Ms. Areen said Dr. Miller's emphasis appears on to be on how the vector is prepared or harvested.

Dr. Anderson said the FDA manual on which Dr. Miller had based his suggestions addresses concerns in preparing biological material on an industrial scale. This manual would not, however, apply to initial human gene therapy research protocols as these procedures will not use materials produced on the industrial scale. Rather, the vectors for human gene therapy will be produced by cultured cells in laboratory-scale tissue culture plates as "budding particles." The budding particle is self-packaged and does not contain culture medium or toxic substances.

Dr. Anderson said human gene therapy protocols will utilize a biological package which excludes contaminants; therefore, Dr. Miller's concerns are not relevant to the points to consider document.

Dr. Anderson said a reference to "toxic chemicals" should not be included in the working group document as experts reading the document would doubt the working group's competence if the points to consider clearly did not apply to human gene therapy procedures.

Dr. Anderson suggested language might be added to the introduction indicating the points to consider document addresses laboratory scale procedures and not industrial manufacturing processes. He volunteered to draft this language.

Dr. Gottesman asked why Dr. Miller was concerned that penicillin or beta-lactam-containing antibiotics might be used in human gene therapy. Dr. Anderson said antibiotics are used in some industrial production processes to protect against bacterial contamination. Antibiotics would not, however, be used in the laboratory scale tissue culture systems producing retroviral vectors for human gene therapy since antibiotics do not control mycoplasma contamination. Indeed, use of antibiotics to control bacterial contamination would be counterproductive since bacterial contamination frequently occurs concomitantly with mycoplasma contamination and can be an indicator of this contamination.

Dr. Walters questioned whether Dr. Miller's concern with product potency, consistency of product lots and product stability were valid. Dr. Anderson said the FDA deals with large-scale systems in which the product is perhaps one part in a million of the culture system. It has not dealt with the system that will be employed in human gene therapy protocols. Product potency is not an issue in human gene therapy except in terms of viral titer. Certainly, there will be no product lot considerations in the initial protocols. In human gene therapy, the cell line producing the retrovirus vector will be constructed in the laboratory and then frozen. When budding particles containing the retrovirus vector are needed, cells will be thawed, plated out in tissue culture medium, and used. The viral particles will bud from the tissue culture cells. Bone marrow cells will be taken from the subject and introduced into the tissue culture medium. The bone marrow cells, which float in the medium, will be exposed to the virus particles. The bone marrow cells will then be harvested and introduced into the patient. There is, thus, no product in the FDA sense. The questions Dr. Temin drafted for Section I-B-2-c-(b) of the document are exactly the questions which should be posed for retroviral vectors produced by budding particles. These questions address the issue in great detail and are more than adequate.

Dr. Murray said Dr. Miller is not familiar with this area. His experience appears to be in the area of drugs and biologicals, and he continues to think of human gene therapy in those terms.

Dr. Walters called the attention of the subworking group to Dr. Miller's comment on Section I-B-2-a-(2) of the working group document. Dr. Miller felt the term "adequate" as used in this section is vague and undefined, and he suggested the section be revised.

Dr. Gottesman suggested "adequate" might be defined in terms of the insertion frequency of the retroviral vector in bone marrow cells. Dr. Anderson felt the current language is acceptable, but said he would draft a clause to clarify

the intent and base "adequacy" on vector insertion frequency in bone marrow cells.

Dr. Walters said Dr. Miller's next comment suggests language be added to Section I-B-2-c; the new language would request a description of previously-reported similar human studies and results (including foreign studies).

Dr. Anderson said to date no similar human studies have been performed. Dr. Gottesman agreed but suggested that if it takes the working group another year to get organized, this type of informational requirement might become pertinent. She thought Dr. Miller's suggested language might be added to the working group document but should not be highlighted.

Dr. Murray felt investigators would include information on similar studies in their proposals regardless of whether or not the working group requests it. Such information would support the proposals. Ms. Areen felt requesting this information would not detract from the points to consider document and suggested it be added even if it does not appear pertinent at this time.

Dr. Walters suggested such language might be added to Section I-B-3 of the points to consider document and volunteered to draft the language.

Dr. Walters called the attention of the subworking group to Dr. Miller's comment on Section I-B-2-c-(1)-(c); Dr. Miller suggested the word "tumor" be substituted for the word "malignancy." Dr. Miller also suggested the language of this section be relocated to Section I-B-2-c-(1) as it is relevant to gene therapy whether or not a retroviral vector is employed.

Dr. Murray said substituting the word "tumor" for "malignancy" will narrow the scope of the question. He said the word "malignancy" includes leukemias as well as tumors. The subworking group agreed the section as written is adequate, and its position in the document is correct.

Dr. Walters called the attention of the subworking group to the comment by Ms. Terri Goldberg, the Executive Director of the Committee for Responsible Genetics, on Section I-B-2-c-(1)-(c) of the points to consider document. Ms. Goldberg said the failure of retroviruses to exhibit oncogenicity or other pathogenicity in laboratory tests is no guarantee they will not become novel human pathogens.

Dr. Anderson said the points to consider document requests test data to address this issue. Dr. Walters said for any therapy risk must be balanced against benefits; for genetic diseases untreatable by any other therapy the projected benefit must be weighed against the risk.

Dr. Walters then called the attention of the subworking group to Dr. Miller's comment on Section I-B-2-c-(1)-(d) of the points to consider document. Dr. Miller had suggested the phrase "specifically germ line cells" be deleted because concern about effects on gametes would be no greater than concern for

other tissues. Dr. Miller wrote the section might request information on "the specificity of transformation or other effects on the target cells."

Drs. Anderson and Gottesman said they preferred to leave the phrase "specifically germ line cells" in the points to consider document. The subworking group agreed.

Dr. Walters called the subworking group's attention to Dr. Miller's comment concerning Section I-B-2-c-(1)-(e) of the points to consider document. Dr. Miller had suggested the first sentence of this section be revised to read: "Describe animal experiments completed or in progress employing protocols similar to that proposed."

Dr. Anderson said Dr. Miller had not grasped the importance of primate studies; these very important studies should be emphasized in the document because endogenous primate retroviruses differ from those in mice or other animals. He felt very strongly that this language should be in the document.

Dr. Walters said Dr. Sheldon Horowitz of the University Hospital and Clinic of the University of Wisconsin-Madison, Madison, Wisconsin, had commented that prior primate testing should not be required in urgent cases. Dr. Horowitz said he personally was treating a young girl with ADA deficiency and severe combined immune deficiency who should receive gene therapy as soon as possible. No other therapy is possible for this patient. He felt he had about 12 months in which to treat this child. The urgency of this case would not leave sufficient time to perform primate testing.

Ms. Areen said the information requirements in the points to consider document are flexible; the document simply states the type of information the working group wishes to review. Decisions would be made on a case-by-case basis.

Dr. Anderson said the document was not requiring a priori that primate testing be performed prior to human gene therapy. The language of this section had been carefully drafted to simply inquire whether primate testing has been done without requiring it for approval.

Dr. Gottesman agreed primate testing is not an a priori requirement; she suggested the document emphasize the flexibility of the points to consider. Dr. Walters suggested the points to consider document should explicitly state that proposals would be reviewed on a case-by-case basis. Dr. Childress thought the working group might also indicate the rationale for developing a "points to consider" document.

Dr. Walters said Dr. Miller's next comment was that the criteria for selection of subjects should be included in Section I-B-3 rather than in Section I-C of the document.

Dr. Walters said the selection of subjects issue has two components. These are: (1) who receives a scarce resource if several people are competing for access; and (2) how subjects are chosen as part of the research design in a large randomized clinical trial. Dr. Walters said Section I-C addresses the first of these issues rather than the second. The subworking group agreed the position of this section in the working group document was correct.

Dr. Gottesman called the attention of the subworking group to Dr. Miller's comment on Section I-B-2-c-(2). Dr. Miller had suggested the following question be included in this section: "How long have animals - especially those in which the desired gene is retained and/or expressed - been followed after treatment?"

Dr. Anderson felt investigators would supply this type of information, but agreed the question could be explicitly stated in the document.

Dr. Walters called the attention of the subworking group to Dr. Miller's next comment. Dr. Miller had suggested the first sentence of Section I-B-3-e is sufficient to convey the intent; the other two sentences require extremely complex speculation of the investigator and should be deleted.

Dr. Anderson said every clinician performs this type of complex speculation everyday; there is nothing unusual in this request. Dr. Walters said the working group would like an indication that investigators had considered contingencies should the procedures not produce the expected results.

Dr. Walters then called the attention of the subworking group to the comment of Dr. Robert McKinney of the National Institutes of Health. Dr. McKinney suggested Sections I-B-3-f, I-B-3-g, and I-B-3-h should be placed in a separate section entitled "Reporting Requirements."

The subworking group agreed this was a reasonable suggestion.

Dr. Walters then asked the subworking group for its opinion on Dr. McKinney's suggestion that the words "are to" should be substituted for the word "should" in the current Sections I-B-3-f and I-B-3-g (Attachment II). Dr. McKinney felt the words "are to" are more forceful than the word "should."

Ms. Areen and Dr. Gottesman felt the word "should" was sufficiently forceful. Although the subworking group did not disagree with Dr. McKinney's suggestion, they did not feel substitution of the words "are to" would increase the force of the statement.

Dr. Walters then asked the subworking group for their opinion concerning Dr. McKinney's comment on Section I-B-3-h. Dr. McKinney had suggested this section be amended to require the submittal of autopsy findings as a separate report.

Dr. Murray said an autopsy is generally considered the final report and usually verifies the diagnosis. He felt the working group would definitely wish to receive the autopsy report. Dr. Walters felt autopsy results would influence future decisions of the working group and the RAC.

Dr. Gottesman agreed the intent of current Section I-B-3-h as written is not clear. She suggested information requests dealing with autopsies be posed in two different sections of the points to consider document: (1) the section dealing with research design, and (2) the section on reporting requirements.

Dr. Walters called the attention of the subworking group to the comments submitted by Mr. Timothy J. McDermott of Mount Auburn Hospital of Cambridge, Massachusetts. Mr. McDermott suggested the danger to the phlebotomist from accidental needle puncture (self-innoculation) should be dealt with in a straightforward manner and health care workers should be closely monitored. He also suggested human gene therapy subjects should be prevented from ever donating blood.

Dr. Anderson thought these issues are public health considerations and adequately covered in the points to consider document. He said the vector to be used in human gene therapy is a very debilitated retrovirus which is not infectious and should not present a potential danger to health care personnel. The subworking group agreed a specific prohibition against gene therapy recipients donating blood might stigmatize these individuals.

Dr. Walters then called the attention of the subworking group to Dr. McKinney's comment on Section I-B-5-a of the document. Dr. McKinney had suggested this section should reference Section III-D. The subworking group agreed Section I-B-5-a should reference Section III-D.

Dr. Walters noted that Dr. McKinney had also suggested Section I-B-5-b be amended to request information on all facilities in which either primary and/or follow-up studies will be performed since these care facilities may not be the treatment facility.

Dr. Anderson agreed this might be a reasonable information request since the patient could be transferred to a nursing home for follow-up care. The subworking group agreed to include this specification in the points to consider document.

Dr. Walters said Dr. Leon Kass of the National Humanities Center in Research Triangle Park, North Carolina, had commented (Attachment V) that the words "equity consideration" of Section I-C are too abstract and remote. Dr. Kass suggested the sentence might read: "How will patients fairly be chosen, if there are more who need treatment than can be treated?"

The subworking group noted that many people think there will be a scarcity of patients for initial human gene therapy protocols but agreed the language proposed by Dr. Kass was clearer than the language currently in the points to consider document. They agreed to substitute Dr. Kass' proposed language in Section I-C.

Dr. Walters then asked the subworking group to consider Dr. Miller's comments concerning Section I-D. Dr. Miller wrote that points 1 through 5 are only a partial listing of informed consent requirements and are thus misleading and should be deleted. If a listing of informed consent requirements is deemed necessary, it should include all the elements of informed consent contained in the regulations. Dr. Miller suggested the initial paragraph under "informed consent" alone would provide sufficient guidance if the following language were added to the paragraph:

"The consent form must adhere to the requirements of 45 CFR 46 and 21 CFR 50. Special attention must be paid to the applicability of the additional elements of informed consent listed in these regulations. (Include a copy of the patient consent form as part of the documentation requested in Part III, below.)"

Dr. Walters felt after events such as Baby Fae, he was inclined to believe these specific points were worth emphasizing. Ms. Areen suggested a sentence to that effect might be added to the preamble of Section I-D. The subworking group agreed, however, that the points to consider document should request the patient consent form as part of the documentation.

Dr. Walters then called the attention of the subworking group to Dr. Miller's comment on Section I-E of the points to consider document. Dr. Miller thought the initial paragraph under Section I-E, Privacy and Confidentiality, provides sufficient information; he suggested Sections I-E-1 and I-E-2 be deleted from the document.

Dr. Childress disagreed with this suggestion; he felt these specific points should be mentioned. Ms. Areen agreed with Dr. Childress. Dr. Walters thought the first patient to receive human gene therapy would be a celebrity; the patient's patient's family should be aware of the difficult issues associated with medical firsts. The subworking group agreed Section I-E as written should remain in the document.

Dr. Walters then called the attention of the subworking group to Section II of the points to consider document. He said several comments were received on this section of the document.

Dr. Walters said Dr. Miller had commented on Section II of the document. Dr. Miller wrote he strongly believed Section II-A was the only point in Section II of the document which should be retained. The other sections are not appropriate to this document or are duplicative and should be deleted. He argued inclusion of these points reflects incomplete understanding of the concept that somatic cell gene therapy is analogous to other conventional medical interventions.

Dr. Walters called the attention of the subworking group to the comment from Dr. Eric T. Juengst of the University of California, San Francisco. Dr. Juengst suggested some of the questions posed in Section II-C, Social Issues, should be moved to Part I, Section A, Objectives and rationale of the proposed research, of the document. Dr. Walters said Dr. Juengst had commented that if the working group wishes to encourage researchers to think about Section II-C questions as an integral part of planning their own work and not as extraneous social kibbitzing, these issues might be worked into particularly relevant points in the document.

Dr. Walters called the attention of the subworking group to the comments of Dr. Leon Kass on Section II. Dr. Kass said Section II-C as written suggests some members of the working group are not entirely comfortable with the distinction between somatic cell therapy and germ line therapy. He argued that gene therapy techniques for somatic cells will almost certainly render germ line genetic

manipulation more possible technically. For this reason, he preferred each submitter be asked to respond directly to the question of whether and how the proposed work might make more feasible the possibilities listed in Sections II-C-3-a and II-C-3-b.

Dr. Walters said Dr. Maxine Singer of the National Institutes of Health had also commented on Section II-C; she thought the first question of this section is a scientific and medical issue and should not be posed among the social issues. The questions raised in Sections II-C-2 and II-C-3 should be considered by investigators as well as other interested individuals. But these questions are not amenable to scientific analysis, and the answers will be diverse and reflect the values of the respondent. Since they are essentially political in nature, these sections will be a source of contention under all circumstances and likely to be very troublesome in the review and approval of proposals. In addition, the context in which these questions appear is unusual; the document suggests RAC will expect written consideration of these questions but response is at the discretion of the investigator.

Dr. Walters said Dr. Juengst had also written that Section II-C-2 of the document is not clear. Are applicants to respond in terms of existing methods of care for the particular disease, or are they to draw analogies to health care in general? If the point is to have the applicants think about how their procedure fits into the traditions and norms of current health care practices, it might be more effective to ask explicitly for analogies; i.e., to have the applicants detail the similarities and differences they see between traditional modes of therapy and human gene therapy procedures.

Dr. Gottesman agreed Section II-C-1 poses a primarily scientific question; she suggested this issue might be highlighted in Part I of the points to consider document under the section discussing the advantages of various therapies. Dr. Anderson said Section I-B-2-c-(1)-(d) addresses this type of issue.

Dr. Murray said while highlighting these issues in Part I of the document was an acceptable approach, the emphases of the different parts of the document are different. Part I of the document asks what is the evidence the proposed somatic cell therapy will not affect the reproductive cells of the patient; Part II of the document is directed towards discussion of social issues and values.

Dr. Anderson agreed Part II of the document attempts to inform the public and the investigator of the types of social issues the RAC and working group will discuss during reviews.

Dr. Walters said Dr. Juengst had commented that if the document indicates investigators can comment on an issue at their discretion, they will undoubtedly submit some comment simply because they feel they must. Dr. Gottesman said the working group and RAC would not wish to see reams of irrelevant information simply because the investigators feel they must comment. She felt the sub-working group agrees the issues raised in Section II-C should be discussed during protocol review. She said she would attempt to compose some language for the introduction which would suggest the working group will consider these

issues; investigators may comment if they feel they have something to add to the discussion.

Dr. Childress said he would to attempt to draft language to discuss the function of Part II of the document. This language would be inserted in the introduction of the points to consider document.

Dr. Walters asked whether Section II-C-3 which asks whether somatic cell therapy will lead to germ line therapy should be deleted since this section had taken the greatest amount of flack from commentors.

Dr. Anderson felt this issue should be mentioned in the points to consider document since RAC will discuss such issues. Dr. Gottesman said the question is whether the RAC or the working group must have an answer to this question before recommending approval of a proposal. Ms. Areen suggested a question could be added to the document inquiring whether this outcome would be "good" or "bad" for society.

Dr. Murray said most clinicians agree human gene therapy is no different from other forms of health care whose primary aim is to help the patient. Medical ethics suggest investigators should, thus, proceed with this form of therapy. Issues may exist but recognition of these issues should not block application of this therapy.

Dr. Anderson felt the words "will lead to" in Section II-C-3 bias the respondent, and should be modified.

Ms. Areen said Dr. Kass specifically asks whether somatic cell therapy renders more technically feasible germ line manipulation for the purpose of enhancing characteristics. She thought this issue should be included in Part II of the points to consider document since it is primarily a social issue in spite of the technical considerations.

Dr. Anderson thought the initial human gene therapy protocols would not produce technical information which might be applied to enhance multigene characteristics. He did not wish to include a statement in Section II-C-3 discussing such possible ramifications of somatic cell gene therapy if initial protocols will not generate such data.

Dr. Gottesman suggested Dr. Kass' question and the considerations raised by Dr. Anderson should be discussed in the introduction of the points to consider document.

Dr. Walters suggested the document's introduction should contain language indicating that on the basis of the report "Splicing Life," the recent Office of Technology Assessment (OTA) report entitled "Human Gene Therapy," and the working group's own discussions, the working group has arrived at the consensus that human gene therapy is no different than other types of therapy. The approach of the points to consider document presupposes this consensus. Dr. Walters offered to attempt to write language to this effect for inclusion in the introduction.

The subworking group agreed Sections II-A and II-B would remain in Part II of the points to consider document. The issues addressed in Section II-C would be discussed in the introduction to the document with emphasis placed on scientific safeguards and patient benefit.

Dr. Walters called the attention of the subworking group to Part III of the document. The subworking group agreed this portion of the document as written was adequate.

Dr. Walters said Mr. Robert Mitchell, the Chair of the RAC, had asked the subworking group to discuss whether the Working Group on Human Gene Therapy should evaluate germ line gene therapy with particular emphasis on the issue of enhancement of characteristics. The working group would function as a study group in evaluating these issues. If the working group agrees to accept this responsibility, the proposed directive would go to the RAC for approval since such discussions are not part of the original mandate of the Working Group on Human Gene Therapy.

Dr. Walters felt this discussion would be a reasonable approach since the working group would not wish to be in the posture of not having evaluated these issues when the first somatic cell gene therapy proposals are submitted.

Dr. Anderson felt germ line gene therapy protocols would not be submitted to RAC in the near future. At present, no investigator has even begun to work on the techniques necessary for human germ line therapy. He said the data generated using mouse models suggest the high degree of difficulty associated with germ line modifications; a large percentage of the few embryos surviving microinjection of the recombinant DNA suffer lethal mutations.

Dr. Gottesman said although germ line applications are not possible in the near future, the working group might begin to discuss the issues associated with these applications. As part of that discussion, the working group could evaluate the state of the art. It could discuss the technical barriers to these procedures, where the technology is, and where it is going. The ethical problems presented by germ line therapy will not be resolved by waiting to receive the first germ line therapy protocols. Dr. Gottesman said it would also be nice to have in hand a discussion of how germ line therapy differs from somatic cell therapy when the first somatic cell gene therapy protocol is reviewed. She suggested RAC should be very careful, however, in choosing language for the project's mandate.

Dr. Murray agreed the working group and RAC would have to be careful in constructing the mandate since some individuals might interpret consideration of an issue a form of promotion of that issue. Dr. Gottesman said RAC and the working group might indicate this study is initiated in response to comments received on the points to consider document.

Dr. Walters said several comments had been received on the composition of the Working Group on Human Gene Therapy; he asked the subworking group for their views on this issue.

Dr. Anderson said he was amused by Dr. Miller's comment that the working group should be supplemented with additional specialists in the fields of molecular biology, pharmacology, medical chemistry, and medicine since Dr. Miller had driven one such specialist from the working group and almost succeeded in driving out a second.

Dr. Gottesman thought the working group could utilize ad hoc expert consultants for reviews of specific proposals and circulate the first proposals broadly. Dr. Anderson thought this suggestion a good one; a panel of ad hoc advisors could be selected and individuals from that panel could be asked to participate in reviews of specific proposals. Dr. Gottesman thought the proposals might be circulated to selected panel members during the period prior to the working group meeting but after announcement in the Federal Register.

Dr. Gartland said the review process now appears to require protocols be submitted for review at least five months prior to a RAC meeting. He questioned whether the review time could be shortened.

Dr. Gottesman suggested one Federal Register announcement might be used to announce both working group and RAC review; this could save some lead time.

Dr. Murray did not feel the review process should be curtailed; he felt review is part of an educational process. Dr. Anderson said the review process would not be curtailed if the announcements were combined; only the lead time would be shortened.

Dr. Anderson thought investigators will first submit trial balloon proposals since they will probably expect additional requirements may be placed on the proposal by the Working Group on Human Gene Therapy or the RAC.

Dr. Murray asked whether a resubmitted proposal could receive expedited review. Dr. Gottesman said in the past RAC has taken a full vote on proposals. However, in some cases if explicit questions which would not affect safety in a major way had not been answered, the RAC has authorized a working group to look at the data and recommend approval, if warranted, to the NIH Director.

Dr. Walters called the attention of the subworking group to the comments offered by Ms. Goldberg. Ms. Goldberg said the Committee for Responsible Genetics recommends RAC expand the points to consider document to include questions on the hazards and general desirability of proceeding with the design of gene delivery systems using currently available technology. She also suggested: a risk assessment conference on the use of retroviruses as vectors in human somatic cell gene therapy be convened; the Working Group on Human Gene Therapy involve a larger number of public representatives; the working group urge full public disclosure of the results of all human somatic cell gene therapy risk assessment experiments; and the working group investigate the number of private institutions interested in or planning to engage in research involving human somatic cell gene therapy.

Dr. Murray questioned why the Committee for Responsible Genetics was requesting an evaluation of the general desirability of gene delivery systems; he felt at this time the disarmed retroviral delivery system appears to be the most promising system. The subworking group agreed and felt it was not useful to undertake an evaluation of delivery systems at this time. Dr. Anderson said Ms. Goldberg was asking whether we know enough about this system to utilize it in human gene therapy protocols. He responded that obviously not enough is known about how this delivery system will function; that is why scientists are asking.

Dr. Gottesman thought reasonable a request for a risk assessment conference on oncogenic viruses as well as on retroviral vectors in human gene therapy. Dr. Anderson said he was currently discussing with Dr. Frank Press, the President of the National Academy of Sciences (NAS), potential symposium topics to present to the NAS council at the April 1985 meeting. He said he could propose NAS sponsor an October symposium on these issues. Dr. Gottesman suggested NAS might consider whether germ line therapy issues should be discussed. Dr. Anderson felt such a discussion would be premature at this time.

Dr. Gottesman questioned Ms. Goldberg's definition of "public members;" she noted that several members of the Working Group on Human Gene Therapy are public members. Dr. Anderson felt Ms. Goldberg was referring to members of the Committee for Responsible Genetics when she used the term "public members."

Dr. Murray felt "public members" should be individuals who did not have a political "stake" in the outcome of the debate; the Committee for Responsible Genetics members may have a political stake in the outcome of deliberations. He thought the working group should avoid nominating individuals with a political stake since it would then be obliged to include representatives from every group in the political spectrum. He said working group meetings are open to the public, and the documents developed by the working group are distributed to the public for comment. This process encourages public participation.

Dr. Walters felt some individuals associated with the Committee for Responsible Genetics could contribute to the working group effort. Dr. Anderson suggested these individuals could be appointed to the ad hoc panel of experts the subworking group was proposing. The subworking group supported this suggestion.

Dr. Walters asked why Ms. Goldberg was specifically urging the working group to attempt to have full public disclosure; the working group is attempting to have full public disclosure.

Dr. Walters said Ms. Goldberg's final point was that the working group should investigate private institutions which might be interested in human gene therapy applications. Ms. Areen questioned how the working group could possibly investigate private institutions when the NIH does not have the authority to require firms to report their activities.

Drs. Walters and Anderson said the only industry researcher currently working in human gene therapy is affiliated with Genentech, Inc. This individual will come to RAC as the project was originally supported by NIH funds. Dr. Walters said at this time there does not appear to be any commercial incentive for companies to develop human gene therapy protocols.

Dr. Walters adjourned the meeting of the subworking group at 12:10 p.m.

[Executive Secretary's Note: The version of the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" developed at this meeting is Attachment VI.]

Respectfully submitted,

Elizabeth Milewski

Elizabeth A. Milewski, Ph.D.
Rapporteur

William J. Gartland, Jr., Ph.D.
Executive Secretary

10/1/85
Date

LeRoy Walters

LeRoy Walters, Ph.D.
Chair

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ATTACHMENT II OF THE MINUTES OF THE MARCH 15, 1985, MEETING OF THE SUBWORKING GROUP ON HUMAN GENE THERAPY IS PAGES 2940 TO 2945 OF THE JANUARY 22, 1985, FEDERAL REGISTER WHICH CAN BE FOUND IN VOLUME 9 OF RECOMBINANT DNA RESEARCH.

SUMMARY
PUBLIC COMMENTS ON
"POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF
HUMAN SOMATIC-CELL GENE THERAPY PROTOCOLS"
[Federal Register 50(14): 2940-2945, January 1985]

Introduction

1. Add "and FDA regulations (21 CFR, Parts 50 and 56)" after the DHHS reference. Then clarify next sentence to read "These DHHS regulations", etc.
2. Proprietary info could be disclosed in closed session--note availability.

Miller

Part I

Section A

Move some of the questions from IIC to this section.

Juengst

Section B

1.b. Change "structure" to "composition."

Add two sections--describe details for harvesting, extraction, and purification and removal of toxic chemicals introduced by these procedures; a warning re: penicillin and other beta-lactam-containing antibiotics used in production of materials for patient.

Address questions re: potency of product to treat cells; consistency of product lots and usage; and stability of product (storage).

2.a.(2) Revise--"adequate" vague, and frequency uncertain at first.

2.c. Request results from similar human studies reports here and abroad, with results.

2.c.(1)(c) "Tumors" for "malignancy" and relocate to I.B.2.c.(1)

Miller

2.c.(1)(c) Failure of retroviruses to exhibit oncogenicity or other pathogenicity in lab tests no guarantee that they will not become novel human pathogens.

Goldberg

2.c.(1)(d) Delete "specifically germline cells." How about "the specificity of transformation or other effects on the target cells?"

2.c.(1)(e) Clarify: "Describe animal experiments completed or in progress employing protocols similar to that proposed."

Miller

2.c.(1)(e) Should not require prior primate testing in urgent cases.

Horowitz

---

3. Selection of subjects criteria belong here vs. I.C.

Horowitz

Miller

3.e. Delete all but first sentence--confusing.

Miller

3.f,g,h. Form Part IV: Reporting Requirements.

3.f,g. "Are to" for "should"--more forceful.

3.h. Request sumbission of autopsy report separately.

McKinney

4.b. Patients treated should never donate blood--code with Red Cross.

4.c. Emphasize potential danger to phlebotomist.

Closely monitor all involved health care workers.

McDermott

5.a. Add cross reference to III.D. at end.

5.b. Amend to include all facilities, including primary and follow-up.

McKinney

Section C

Move section to I.B.3.

Miller

Section D

Points 1-5 are misleading and should be deleted. Add the following instead: "The consent form must adhere to the requirements of 45 CFR 46 and 21 CFR 50. Special attention must be paid to the applicability of the additional elements of informed consent listed in these regulations. (Include a copy of the patient consent form as part of the documentation requested in Part III, below.)"

Miller

Section E

Delete points 1 and 2 under "Privacy and Confidentiality."

Miller

Part II

Section A

Only this part should be kept.

Miller

Section C

1. Not social but medical/scientific--improper.

Singer

Already answered previously.

Juenqst

---

2. Written consideration of question should not be required.

Singer

Confusing--just note similarities and differences.

Juengst

---

3. Is investigator a soothsayer? Several aspects are political and therefore contentious and trouble spots.

Singer

Integrate questions at other points in the document.

Juengst

This section pertains to general vs. the case approach and should be considered by public before the therapy is undertaken. Since the RAC doesn't seem to have a position on the positive and negative aspects of gene therapy, it is hard to review protocols. The document fails to answer the President's Commission's report on gene splicing that you were formed to answer.

Cavalieri

Part III

F and G request the same information.

McKinney

General Comments

Clinical trials of gene therapy in life-threatening cases should be permitted to begin now. The government should not stand in the way of parents' making "reasonable choices" for the lives of their children.

Horowitz

The NIH guidelines should be extended for germline cells. Also, the professional composition of the Working Group should be expanded to include more social scientists and theologians. More attention should be paid to the profound ethical and psychological issues raised by both somatic-cell and germline gene therapy.

Rifkin

The Working Group should be supplemented with additional specialists in the fields of moecular biology, pharmacology, medical chemistry, and medicine. Chair--should be a scientist, preferrably a physician. Most decisions on individual protocols will be scientific and medical.

Miller

The Group needs to address:

The potential public health impact of using new techniques with current technological levels, etc.--appropriate technology.

The role of public participation in the safety and desirability of somatic cell gene therapy experiments--care has been shown outside.

The oversight of non-NIH funded institutions that want this therapy. Should there be some public service for the private?

Goldberg

The interrogative style of the document could be interpreted as strident or adversarial. Rationale not provided for many of the questions. Need a more explicative, collaborative approach like that embodied in FDA's draft "Points to Consider in the Production and Testing of New Drugs, and Biologicals...."

Miller

Need risk assessment conference on the use of retroviruses as vectors in somatic-cell gene therapy.

Goldberg

Increase number of public representatives on the Working Group from 1 to 3. Promote public discussion of risk assessment questions regarding retroviral vectors.

Goldberg

Working Group should explore how many private firms not falling under NIH Recombinant DNA Research Guidelines will become involved in gene therapy, report findings to the Congress and the public.

Goldberg

Since the RAC and its Working Group have not responded to all of the issues raised by the President's Commission, "it is premature to consider a review process for individual proposed human gene therapy protocols."

Cavalieri

Add a section at the end of the document such as the following: "For information on the jurisdiction of the Food and Drug Administration (FDA) over products employed for human gene therapy, please refer to FDA's "Statement of Policy for Regulating Biotechnology Products," Federal Register 252, 50878, 1984.

Miller

Prepared by LeRoy Walters with the assistance of Heidi Wiljanen. 3/14/85

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Bethesda, Maryland 20205
Building : 31
Room : 3B10
(301) 496- 6051

March 7, 1985

M E M O R A N D U M

To: Members
Working Group on Human Gene Therapy

From: Executive Secretary

Subject: Comments

Attached are the comments received to date on the Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols published in the Federal Register on January 22.

William J. Gartland, Jr., Ph.D.

Attachment

Addressees:
Dr. Anderson
Ms. Areen
Mr. Capron
Dr. Childress
Dr. Gorovitz
Dr. Gottesman
Dr. Grobstein
Dr. Mahoney
Mr. Mitchell
Dr. Motulsky
Dr. Murray
Dr. Rich
Dr. Varmus
Dr. Walters
Ms. Witherby
Dr. Temin
Dr. Harmison
Dr. Miller
Ms. Lee

cc: Dr. Cook-Deegan

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

National Institutes of Health
National Cancer Institute
Bethesda, Maryland 20205

February 28, 1985

Dr. William J. Gartland
National Institute of Allergy and
Infectious Diseases
National Institutes of Health
Bethesda, MD 20205

Dear Bill:

I write to comment on the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" published in the Federal Register on January 22, 1985. First, I would like to congratulate the working group on a very thorough and thoughtful job. The scientific questions are dealt with carefully and sensibly. Second, I have some questions regarding part II, Social Questions. Subparts IIA and IIB are straightforward. Subpart IIC is problematic. Question 1 is fine, but it belongs among the scientific and medical issues, not among the social issues; to place it here in part II implies questions about values, which in fact are not part of Question 1 at all, as it is stated. I agree that the other two questions raised (IIC2 and IIC3) should be considered by the people doing the experiments as well as others. However, the context here is unusual. The "Points" seems to say that the RAC will expect written considerations of these points, at the discretion of the investigators. Yet, they are not really amenable to scientific analysis; the answers will be diverse and reflect the values of the respondent. Question(s) 3 are the most troubling. While they raise interesting matters for discussion, they ask the investigator to be a soothsayer. Also, several aspects of this question are essentially political in nature. They will be contentious under all circumstances, and likely to be very troublesome in the context of the review and approval of proposals. If the working group wants the IBCs and investigators to think about these matters, they should say so. They should remove their request that the parties discuss these matters in writing in the documents submitted for review. Leaving it to their 'discretion', as stated in the introduction to Part II, implies that the RAC expects this. The questions in II 2 and 3 C are better left to lengthy and public analysis by thoughtful and competent people..... in articles and books.

Sincerely yours,

Maxine Singer

Center for Health Sciences
University of Wisconsin-Madison
University Hospital and Clinics

600 Highland Avenue
Madison, Wisconsin 53792

February 21, 1985

Leroy Walters
Director
Kennedy Institute of Ethics
Georgetown University
Washington, D.C.

Dear Mr. Walters:

I am pediatric immunologist at the University of Wisconsin. I am writing to you about "Points to consider in the design and submission of somatic-cell gene therapy protocols" that was published in the January 22, 1985 Federal Register.

I will restrict my comments to gene therapy for adenosine deaminase (ADA) deficiency. I have personally cared for six children with ADA deficiency. Currently available therapies for ADA deficiency include: 1) enzyme replacement therapy (red blood cell transfusions); 2) thymic factor and/or thymic transplant; 3) bone marrow transplant. Although the first two therapies are usually ineffective, bone marrow transplantation may cure ADA deficiency.

I am now taking care of a 6 1/2 year old child with ADA deficiency and severe combined immune deficiency who I feel should receive gene therapy as soon as possible. Enzyme replacement therapy, thymic factor and thymic transplant have been tried in this child without success. A bone marrow transplant could be tried in this girl. However, since there is no sibling who is identical, it would be a mismatched transplant. In this child, a mismatched bone marrow transplant would be very difficult since the minimal immunity she has would have to be "wiped out" with chemotherapy and radiotherapy prior to transplantation. Also, her chronic lung disease could be significantly worsened by the chemotherapy and radiotherapy. I think it is very likely that the transplant attempt would kill her. Both the parents and I feel that the known risks of bone marrow transplantaton in this child (infection, graft versus host disease, lung toxicity, cancer) are greater than the potential risks of gene therapy. Her lung disease may become too severe if we continue to wait - I feel we have approximately 12 months to do something.

I believe that the risk of the viral vector (retrovirus) combining with viruses in the patient to form a new infectious agent is remote. I would urge your committee to recommend initiating clinical trials of gene therapy in patients with life-threatening illnesses such as ADA deficiency as soon as possible. I feel that clinical trials should not wait for the results of non-human primate studies.

Leroy Walters
February 12, 1985
Page Two

Finally, the parents are very well informed of the issues and very much want to proceed with gene therapy. Their daughter is going to die. There is no reasonable alternative. Gene therapy may have only a small chance of success, but its risks are minimal compared with certain death. I do not believe the government should stand in the way of parents making reasonable choices. It goes without saying that the government is already regulating such choices through the Institutional Review Boards, a process which I strongly support in theory and on its record.

In summary, I feel that clinical trials of gene therapy in diseases such as ADA deficiency should begin as soon as possible.

Sincerely,

Sheldon Horowitz

Sheldon Horowitz, M.D.
Associate Professor of Pediatrics
Division of Immunology

SH:lk

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

National Institutes of Health
Bethesda, Maryland 20205

January 30, 1985

Dr. William Gartland, Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20205

Dear Bill:

This is in response to the request for comment on the "Points to Consider in the Design and Submission of Human Somatic Gene Therapy Protocols" which appeared in the Federal Register, January 22, 1985.

The comments I offer are as follows:

1. Re: paragraphs I.B.3.f, I.B.3.g. and I.B.3.h.

   It is suggested that a new section i.e. IV - Reporting Requirements be created and these paragraphs be incorporated under the new section.

   The cited paragraphs cover information that would not be available at the time of initial submittal and therefore, would be best separated from that body. Additionally, separating these paragraphs under the proposed section would clearly identify the future requirements.

   It is also suggested that the word "should" in the second line of I.B.3.f. and I.B.3.g. be replaced with the words "are to." The word "should" tends to be permissive.

   Lastly, it is suggested that paragraph I.B.3.h. be amended to require the submittal of autopsy findings (if an autopsy is performed) as a separate report.

2. Re: paragraph I.B.5.a.

   It is suggested that a cross-reference to paragraph III.D be inserted at the end after the word "vitae."

3. Re: paragraph I.B.5.b.

   It is suggested that this paragraph be amended to include all facilities at which either primary treatment and/or follow-up

studies will be performed since it may be that these would be different.

4. Re: paragraphs III.F and III.G.

   It is suggested that these be combined since they appear to be requesting the same information.

If you desire clarification of my suggestions, please contact me.

Sincerely,

Robert W. McKinney, Ph.D.

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William Winpisinger
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*Executive Council

Terri Goldberg
Executive Director

February 19, 1985

Dr. William Gartland
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, MD 20205

Dear Dr. Gartland:

I am writing on behalf of the Executive Council of the Committee for Responsible Genetics to comment on the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" published in the Federal Register on January 22, 1985. We believe that the points listed in the document should be expanded in the following areas: potential public health impacts of gene delivery systems using currently available technology; public participation in debates concerning the safety and desirability of proposed somatic-cell gene therapy experiments; and oversight of private institutions, which do not receive NIH funds, that may be interested in or planning to undertake somatic-cell gene therapy experiments.

Risk assessment for recombinant DNA activities has proceeded up to now under the assumption that recombinant organisms would be no more hazardous than any of their constituent parts. However, it is now recognized that placing a normal gene into a relatively innocuous virus can create a tumor virus. We, therefore, urge the Recombinant DNA Advisory Committee to reconsider whether the existing technology is appropriate for the design of somatic-cell gene therapeutic agents, since it is not difficult to devise scenarios whereby such agents may prove hazardous to the public health.

Although human somatic-cell therapy is in some sense analogous to other experimental treatments of individua consenting patients, it differs from such treatment in at least one important way. As discussed in the "Point to Consider," gene therapy necessitates the development of gene delivery systems capable of introducing human genes into human cells. This system may be an infective agent, such as a modified retrovirus. If such an

agent were to prove hazardous, the risk would be borne by individuals other than the patient alone. This is a characteristic not shared by other therapeutic protocols, even those using chemical agents.

Planned gene delivery systems, particularly those using retroviral vectors, may present a danger to the public health. Our concern is based on recent work on animal oncogenic retroviruses. Such viruses can transform normal cells into malignant cells of their hosts by a number of means including the introduction into those cells of a normal or slightly altered copy of a host gene. When this results in quantitative, qualitative, or temporally aberrant expression of the gene's protein product, a tumor can result. So far we have no way of predicting which host-like genes can act as oncogenes when presented to a cell out of normal physiological context, but the list currently includes genes specifying secreted growth factors, plasma membrane and cytoplasmic growth factor receptors, in addition to other common cellular proteins. It is presumed that animal retroviruses have picked up host genes in the course of infection during their evolutionary histories. If so, this must be a relatively rare event which would not necessarily render the recombinant virus oncogenic. Subsequent evolutionary events, such as an alteration of a single nucleotide in the viral version of the host gene leading to an amino acid change in the corresponding protein and possible oncogenicity, would occur more rapidly. It is well known that oncogenic retroviruses in animals can be transmitted "vertically" or "horizontally," that is between infected parents and offspring and from one individual to another within a population.

Although no human retroviruses have yet been described that stimulate tumor formation by means of a host-like viral oncogene, there is no reason in principle why such agents could not arise naturally or by design. Perhaps the human species has been fortunate in harboring no retroviruses equipped to capture a genetic message from a host cell; however, genetic engineering could readily circumvent this situation and produce a retroviral-human gene recombinant. Indeed, this would be the desired goal in designing a somatic-cell gene therapy vector. Furthermore, such artificial recombinants might contain genes, like those for growth factors, that fall into both "therapeutic" and "oncogenic" categories. The failure of such recombinant retroviruses to exhibit oncogenicity or other types of pathogenicity in standard laboratory tests ("Points to Consider" I.B.2.c. (1) (c)) is no guarantee that such recombinants are not a few short steps from becoming novel human pathogens.

Although gene replacement therapy may ultimately prove to be an important tool in the management of certain serious diseases, there are few such diseases for which it is the only conceivable approach. Our concern is that existing technology applied to this end may raise more problems than it solves. We call on the Recombinant DNA Advisory Committee to convene a risk assessment conference on the use of retroviruses for vectors in somatic-cell gene therapy

experiments to determine the potential health effects on the individual and the public.

As was well documented in the Splicing Life report by the Presidential Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, the public has a significant interest in the ethical, social and medical aspects of human gene therapy. We believe that the questions raised in this letter warrant a wider public discussion than that incorporated in current procedures. The Committee for Responsible Genetics urges the Recombinant DNA Advisory Committee to expand the ability of the public to participate in the current debate concerning safety and desirability of proposed somatic-cell gene therapy experiments. We recommend that the number of public representatives on the Working Group on Human Gene Therapy be increased from one to three and that the results of somatic-cell gene therapy risk assessment experiments be made public in scientific journals as well as publications written for the lay public.

There are a growing number of privately-financed medical institutions which may be interested in experimenting with human somatic-cell gene therapy. These institutions would not be under the jurisdiction of the Recombinant DNA Advisory Committee, and therefore there would be no public oversight of their research activities. We urge the Recombinant DNA Advisory Committee to determine the number of private health care institutions which might engage in research involving somatic-cell human gene therapy so that the public and Congress can consider whether there is a need to establish a public body to oversee such research.

In summary, the Committee for Responsible Genetics recommends that the Recombinant DNA Advisory Committee expand the "Points to Consider" document to include questions concerning the hazards and general desirability of proceeding with the design of gene delivery systems using currently available technology, convene a risk assessment conference on the use of retroviruses for vectors in human somatic-cell gene therapy experiments, involve a larger number of public representatives in the Working Group on Human Gene Therapy, urge full public disclosure of the results of all human somatic-cell gene therapy risk assessment experiments, and investigate the number of private institutions which may be interested in or planning to engage in research involving human somatic-cell gene therapy.

Sincerely,

Terri Goldberg

Terri Goldberg
Executive Director

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO SANTA BARBARA • SANTA CRUZ

DIVISION OF MEDICAL ETHICS
SCHOOL OF MEDICINE
DEPARTMENT OF MEDICINE

SAN FRANCISCO, CALIFORNIA 94143

Dr. William J. Gartland,
Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, MD 20205

Dear Dr. Gartland:

I am writing in response ot the request by the RAC Working Group on Human Gene Therapy for comments on th "Points to Consider" published in the January 22 Federal Register. These guidelines represent a commendable effort on the part of the Working Group to prepare for the first human gene therapy protocoals, and for the most part they are clear and thoughtful. I would like comment on one section, hough, that did leave me bewildered, and might merit further attentio:

The onfusing section for me is point C of part II, "Social Issues." This section is potentially one of the most important parts of the document, given the issues it raises, but its usefulness in its present form is blurred in several ways.

First, it is not clear how prospective applicants are to take this section in planning their proposals. They are requested "to discuss, at their discretion, the general issues enumerated in point C" [p.2944]. This discretionary freedom is presumably derived from the earlier acknowledgement that "not every point mentioned in the document will necessarily require attention in every proposal" [p.2942]. Yet, under point C itself they are explicitly advised that "the following issues will also be considered by the RAC and the working group in reviewing each gene therapy proposal" (emphasis added) [p.2944]. I think this wording would almost always make it a tactical mistake for applicants to exercise their discretionary option to make point C one of those they do not treat in their proposal.

Part of the reason for this is the dynamics of proposal review. One of the lessons I learned from four years as a program officer in a federal grant-making agency (NEH) was that, when faced with a difficult task in weighing the merits of a proposal, the more criteria reviewers can justifiably bring to bear, the finer the discriminations they can make. The psychology, if not the logic, of the situation

makes it inevitable that "suggestions to applicants" will become strict review criteria as decisions grow harder. With the normative emphasis given to this point by its explicit characterization as a set of review criteria, applicants may find it harder to avoid addressing this point than some of the more technical considerations that precede it.

The problem with interpreting point C's questions as mandatory, however, is that it is not clear what they are asking the applicants to discuss. For example, point C-1 has presumably already been answered in the proposal, under I-B-2-c-1-d and c-2 (p.2943). In what sense does it pose a "general," "social" issue "beyond the purview of local IRB's? If the point of this question is simply to reassure people that it will be addressed in the review, it might be made more effectively in other ways.

Similarly, what is question C-2 asking? Every human gene therapy trial being currently considered is both "an extension of existing methods of health care" and "a distinct departure from present treatments of disease." It depends on how narrowly one construes "extension" and "departure." Moreover, are the applicants to respond to this question in terms of the existing methods of care for the particular disease they plan to treat, or are they to draw analogies from health care in general, as the wording here suggests? If the point is to have the applicants think about how their procedure fits into the traditions and under the norms of current health care practices, it might be more effective to ask explicitly for analogies -- i.e., to have applicants detail the similarities and differences they see between traditional modes of therapy and their own.

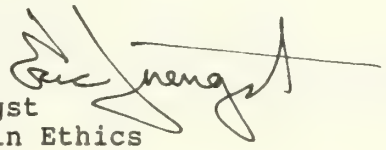
Again, with C-3, are applicants to answer this in terms of their particular project, or, as it says here, for somatic cell gene therapy in general? If it is the latter, what bearing should such an essay have on the review of their particular proposal, other than as evidence of their ability to think and write well about human nature and the social consequences of science? Each of the three questions under C-3 are important ones for the scientific community to be thinking about, and the RAC Working Group can play a vital role in catalyzing that thought. But this approach, packaging them all together at the end of the guidelines in a single vague question to applicants, and leaving unclear the significance of their response to the review of their proposal, seems certain only to be frustrating for scientists.

If the point is to encourage researchers to think about these questions as an integral part of planning their own work, and not as extraneous social kibbitzing, then why not work them into the guidelines where they are each especially relevant? For example, concern that some somatic cell trials will increase the pressure to attempt germ-line therapies is a corrollary of questions I-A-4 [p.2942] about the relative advantages and disadvantages of proposed alternative therapies. Similarly, questions about the distinction between "curing disease" and "enhancing traits" could be considered when discussing the candidacy of the disease selected for treatment,

and what the protocol is designed to accomplish with regard to it (points I-A-1,2,3 [p.2942]). And questions about eventual access policies are logical extensions of the equity issues involved initially in selecting patients for the proposed trials (point I-C-5 [p.2944]). In some ways, these are the issues that scientists and policy-makers thinking about this research need the most help in considering. The Working Group could be very useful in that by discouraging the attitude that they are questions external to and imposed on sound scientific research planning.

I hope these comments are helpful in the Working Group's deliberations. If questions arise about them, please feel free to contact me.

Sincerely,



Eric T. Juengst
Adjunct Lecturer in Ethics

cc. L. Walters

U.S. Department of Labor

Occupational Safety and Health Administration
Washington, D.C. 20210

Reply to the Attention of:

FEB 21 1985

William J. Gartland, Jr., Ph.D.
Director
Office of Recombinant DNA Activities
National Institutes of Health
Building 31, Room 3B10
Bethesda, Maryland 20205

Dear Dr. Gartland:

In accordance with your memorandum of January 22, 1985, the proposed document entitled "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols," has been reviewed and appears to be quite thorough.

Thank you for the opportunity to review this important document.

Sincerely,

Edward J. Baier
Director
Directorate of Technical Support

United States Department of Agriculture

Animal and Plant Health Inspection Service

Washington, D.C. 20250

Dr. William J. Gartland, Jr.
Department of Health & Human Services
National Institutes of Health
Building 31, Room 3B10
Bethesda, Maryland 20205

Dear Dr. Gartland:

Regarding your January 22 memorandum concerning the document entitled "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols," the Animal and Plant Health Inspection Service has no comments on the protocol at this time.

We appreciate the opportunity to review the document.

Sincerely,

James O. Lee, Jr.
Acting Administrator

STANFORD UNIVERSITY MEDICAL CENTER
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOCHEMISTRY

PAUL BERG
Willson Professor of Biochemistry

February 13, 1985

Dr. William J. Gartland
Office of Recombinant DNA Activities
National Institute of Allergy & Infectious Diseases
National Institutes of Health
Bethesda, Maryland 20205

Dear Dr. Gartland,

I am writing to comment on the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" that was published in the Federal Register of January 22, 1985.

I have read the questions that must be addressed by investigators planning genetic modifications of somatic cells for the treatment of certain human genetic diseases. They appear eminently reasonable, specific and thorough. I am certain that responding to the "points to be considered" will greatly aid the scientists and physicians to improve the quality of the protocols and outcomes of such contemplated therapies.. It will certainly help the Federal agencies charged with reviewing and evaluating the merit of such proposed therapies. Moreover, the procedures outlined should also assure public confidence that such procedures are being undertaken with the utmost of care and supervision.

Sincerely,

Paul Berg

PB/e

Comments on the Document "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" published in the Federal Register of Tuesday, January 22, 1985, Part III

TO: Director
Office of Recombinant DNA Activities

FROM: L.F. Cavalieri, Ph.D.
Member, Sloan-Kettering Institute
Professor of Biochemistry, Cornell University
Graduate School of Medical Sciences
Member, Executive Board, Committee for Responsible
Genetics

1. A case-by-case review of proposals for human gene therapy is not appropriate for considering the social issues mentioned in section II-C-3 of the document. These issues apply to all human somatic-cell therapy and therefore require resolution by public consensus before any human gene therapy is undertaken. But as yet, no attempt to reach a conclusion on these issues has been published for public comment.

2. The social concerns listed in sections I-C and II of the document by no means exhaust the issues raised in the "Report on the Social and Ethical Issues of Genetic Engineering with Human Beings" of the President's Commission, to which the RAC Working Group on Social and Ethical Issues was formed to respond.

3. Since the RAC Working Group on Social and Ethical Issues has not yet formulated a position with respect to the positive and negative aspects of human somatic-cell gene therapy raised in the Report of the President's Commission and elsewhere, it is premature to consider a review process for individual proposed human gene therapy protocols.

Dr. L. F. Cavalieri
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Timothy J McDermott
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Boston, MA 02114
617-720-4615

Director, Office of Recombinant
DNA Activities
Building 31, Room 3B 10
National Institutes of Health
Bethesda, MD 20205

February 18, 1985

Dear Sir/Madam:

I am writing to comment on the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" submitted by the working group on Human Gene Therapy of the NIH's Recombinant DNA Advisory Committee, FR Doc 85-1463 Filed 1-18-85. I will refer to the outline provided in the Points to Consider document to identify my specific area of comment.

In section I B 4 c, "What precautions will be taken, if any, to protect others (eg patients sharing a room, health-care workers or family members) from such potential hazards?" , I emphasize that the danger for the phlebotomist from accidental needle puncture (self innoculation) should be dealt with in a straight forward fashion. One model that could be expanded on is the general hospital protocol for screening all accidental needle punctures. The emphasis of these protocols is to identify the hazard (eg is the patient HBsAg - positive) and to provide the best preventitive measures early on for the person who has been accidentally stuck (victim).

In regards to an experimental protocol such as Human Somatic-Cell Therapy I emphasize the prudence of following closely (every 4-6 months) pertinent physiological parameters of the health-care worker(victim) in the best interest not only of that person but in promoting a more thorough understanding of this new therapy.

In section I B 4 b, "Is there any expectation that recombinant DNA will spread from the patient to others or to the enviroment?" I propose that persons undergoing this therapy be prevented from ever donating blood. Some "flag" (eg Social Security #) should be stored within the American Red Cross' blood inventory system so that blood donors having undergone cell therapy will be automatically indentified in routine processing.

Please consider these suggestions in the sincere and constructive spirit in which they were offered.

Sincerely,

Timothy J McDermott MT(ASCP)
Dept of Biochemistry
Mount Auburn Hospital
Cambridge, MA 02238

FOUNDATION ON ECONOMIC TRENDS
1346 Connecticut Avenue, NW, Suite 1010 Washington, DC 20036, (202) 466-2823

February 20, 1985

Director
Office of Recombinant DNA Activities
Building 31, Room 3-B10
NIH
Bethesda, Md. 20205

Dear Director,

The following comments are in response to the NIH guidelines for human somatic gene therapy that was published in the Federal Register on January 22, 1985.

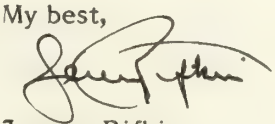
While the NIH guidelines mention the possible germline impact of somatic therapy, too little detail is provided on the profound ethical and physiological issues raised by this potential relationship.

The National Institutes of Health has not devoted enough time and attention to this important consideration. It is our position that the NIH needs to develop more elaborate guidelines that would take into account the possible impact of somatic therapy on germline cells. The NIH regulations contained in the January 22nd Federal Register are woefully inadequate in this regard.

Somatic and germline therapy of human beings represents a qualitative departure in our medical practices as both therapies involve changing basic instructions in the genetic code. The question of engineering changes in the somatic cells or germline cells raises a host of new ethical and public policy considerations that require broader discussion and broader representation than are evidenced in the proceedings of the RAC subcommittee responsible for drawing up these guidelines.

The working group on human gene therapy of the Recombinant DNA Advisory Committee of the National Institutes of Health is not broad-based enough in its professional composition to adequately represent the spectrum of opinion and views that need to be included in any discussion of human gene therapy. For example, the committee has no representation from the major religious denominations. In addition, the academic disciplines represented on the committee are very limited and include only one philosopher and one ethicist. There are no anthropologists, sociologists, psychologists, or theologians, all of whom could make important contributions by bringing to this issue a range of different perspectives which would be important in understanding the full impact of the genetic engineering of somatic cells.

My best,



Jeremy Rifkin

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Bethesda MD 20205

FEB 20 1985

Dr. William J. Gartland, Jr.
Director, Office of Recombinant
DNA Activities
NIAID
NIH
Bethesda, MD 20205

Dear Bill,

The FDA appreciates the efforts of the RAC's Working Group on Human Gene Therapy to draft a document that will provide guidance to clinical investigators about to embark on gene therapy. As you are aware, the FDA has provided similar documents on a variety of subjects and these have, we are sure, expedited the submission and review process.

My colleagues and I have reviewed the NIH's "Points to Consider" carefully, and on the following pages we offer some general and specific comments regarding the document. We believe that the document will require some revision prior to its promulgation in final form and hope that our comments will be helpful. In addition, through our liaison representatives, we look forward to continued collaboration between NIH and FDA in this area.

Sincerely yours,

Henry I. Miller, MD
Food and Drug Administration Liaison
to the RAC

FDA's Comments on NIH's Proposed "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols."

1. In the Introduction on p. 2942, middle column, third complete paragraph: following reference to the DHHS regulations (45 CFR, Part 46), add "and FDA regulations (21 CFR, Parts 50 and 56)." The next sentence would then need to be modified to clarify that "these regulations" refer to the DHHS regulations.

2. In the discussion of the unlikelihood of proprietary information in gene therapy proposals, it should be noted that where proprietary information is included, those portions of proposals would be discussed during closed sessions. While this may not happen frequently, the option for closed sessions should be available.

3. In section I.B.1.b. on p. 2943, we suggest that the wording would be clearer in the first sentence if it were, "What is the composition of the material . . . "

4. In section I.B.1.b on p. 2943, we suggest the addition of two new sections:

   a. One would request the investigator to, "Describe in detail the methods for harvesting, extraction, and purification, and for the removal of any toxic chemicals introduced by these procedures."

   b. The second would contain a warning that penicillin and other beta-lactam-containing antibiotics should not be used in the production of materials administered to patients.

5. In Section I.B.1.b.(1) on p. 2943, we suggest the addition of questions about: (1) the methods for assaying potency of the product used to treat cells or administered to a patient; and (2) the consistency of the product lot-by-lot, if different lots will be used on the same patient or on different patients; and (3) the stability of the product under the projected conditions of storage.

6. In section I.B.2.a.(2) on p. 2943, because it will be difficult to predict the frequency of insertion of the desired DNA sequences into the patient's cells prior to performance of the experiment (particularly if in vivo selection is crucial) and "adequate" is vague and left undefined, this section should be revised.

7. In section I.B.2.c. on p. 2943, we suggest the addition of a section which would request a description of previously-reported similar human studies and their results (including foreign studies).

8. In section I.B.2.c.(1)(c) on p. 2943, in the first sentence, "malignancy" should read "tumors." Moreover, because this section is relevant to gene therapy whether or not a retroviral vector is employed, it should be relocated to above I.B.2.c.(1).

9. In section I.B.2.c.(1)(d) on p. 2943, we believe that the phrase "specifically germline cells" should be deleted because apprehension about an effect on gametes should be no greater than for other tissues including, for example, blood or bone cell precursors. A clearer way of asking the question might be, "What is the specificity of transformation or other effects on the target cells?"

10. In section I.B.2.c.(1)(e) on p. 2943, we believe that the first sentence would be clearer if revised to read, "Describe animal experiments completed or in progress employing protocols similar to that proposed."

11. In section I.B.2.c.(2) on p. 2943, we suggest that an additional question be included, "How long have animals - especially those in which the desired gene is retained and/or expressed - been followed after treatment?"

12. In section I.B.3., on p. 2943, we believe that the criteria for "selection of subjects" should be included here, rather than as a separate section I.C.

13. In section I.B.3.e. on p. 2943, we suggest that the first sentence of the section is sufficient. The remainder requires of the investigator extremely complex speculation and should be deleted.

14. In section I.D.: The initial paragraph under "informed consent" would provide sufficient guidance, without points 1-5, if the following were added to the paragraph:

> "The consent form must adhere to the requirements of 45 CFR 46 and 21 CFR 50. Special attention must be paid to the applicability of the additional elements of informed consent listed in these regulations. (Include a copy of the patient consent form as part of the documentation requested in Part III, below.)"

Points 1-5 are a partial listing of the elements of informed consent and may be misleading to applicants. If such a listing in detail is determined to be necessary, we believe it should include all of the basic and additional elements of informed consent contained in the regulations.

15. In section I.E. on p. 2944, the initial paragraph under "Privacy and Confidentiality" would, we believe, provide sufficient information, without points 1 and 2.

16. In section II on p. 2944, we believe strongly that only point A should be retained. The others are, we believe, not appropriate to this document or are duplicative. The inclusion of the other points would reflect incomplete understanding of the concepts discussed in point #19, below.

17. We suggest the addition of a section at the end of the document such as the following, "For information on the jurisdiction of the Food and Drug Administration (FDA) over products employed for human gene therapy, please refer to the FDA's "Statement of Policy for Regulating Biotechnology Products", Federal Register 252, 50878, 1984.

18. We are concerned that the interrogatory style of the document may be interpreted as strident or even adversary. Moreover, this style does not provide the rationale for many of the questions although arguably the rationales could be invaluable for the formulation of responses. We believe that both the tone of the document and the quality of communication between NIH and investigators would be improved by a more explicative, collaborative approach. As an example of this, Attachment A is the FDA's draft "Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology."

19. We have one additional concern. There exists virtual unanimity among those conversant with human gene therapy that somatic-cell gene therapy is analogous to other conventional medical interventions, such as vaccination, exogenous replacement of insulin in diabetics, etc. The conclusion of the recent OTA Report, "Human Gene Therapy-Packground Paper" is instructive:

> Civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in human for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies.

The FDA has also endorsed this view in principle and has made known its intention to subject appropriate products employed for human gene therapy to an administrative review similar to that for analogous biological drug products. Since as implied above, most of the critical decisions on individual investigators' submissions are likely to be scientific and medical ones, we believe that it is imperative to reconstitute the membership of the RAC Working Group on Human Gene Therapy [which will conduct the initial administrative review of proposals] which currently includes a large proportion of attorneys, ethicists and public policy specialists. The Working Group should be supplemented with more members with expertise in molecular biology, pharmacology, medicinal chemistry, and medicine. Moreover, we believe that the chair should be a scientist, preferably a physician, with expertise in a field closely allied to molecular biology, internal medicine, or genetics.

0405f

Attachment A

DRAFT
NOVEMBER 7, 1983

Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology

Office of Biologics Research and Review

National Center for Drugs and Biologics

Table of Contents

I. Introduction

II. General Considerations

III. Expression System

IV. Master Cell Bank

V. Production

VI. Purification

VII. Characterization of the Product

A. Physicochemical Characterization

B. Biological Tests

C. Tests for Contaminants

D. Toxicity Tests in Animals

VIII. Modified Protein Products

IX. Clinical Trials

I. Introduction

This document provides suggestions for controlling safety, purity, and potency of new drugs and biologics produced by recombinant DNA technology. The suggestions expressed herein are expected to change with time as new knowledge is acquired, and should not be regarded as being either fixed or all-inclusive. Accordingly, the points discussed below should be interpreted as being what manufacturers of such products are generally expected to consider during drug development, in applications for investigational new drug (IND) exemptions, in new drug applications (NDA), and in license applications. The general regulations applicable to other biologics (general safety, sterility, potency, etc.) are also applicable to recombinant DNA biological products. Specific concerns relevant to particular products should be discussed with the appropriate Office on a case-by-case basis.

Although considered voluntary for industry, these points represent an informed consensus relating to the general safe use of products developed using recombinant DNA technology.

New license applications or new drug applications are required for products made with recombinant DNA technology, even if the product is considered to be identical in molecular or chemical structure to a naturally occurring substance or previously approved product produced in a conventional way.

II. General Considerations

The production of new drugs and biologics by recombinant DNA technology should generally follow the NIH Guidelines for Research Involving Recombinant DNA Molecules or other appropriate guidelines.

III. Expression Systems

Recombinant DNA technology involves systematically arranging and manipulating specific segments of nucleic acid to produce a composite molecule which, when placed into an appropriate host environment, will yield a desired product. There are three general methods for obtaining a specific coding segment: (a) reverse transcription of mRNA to complementary DNA; (b) isolation of genomic DNA or RNA; or (c) chemical synthesis as predicted from a peptide sequence.

The manufacturer should provide a description of the method used to prepare the segment coding for the desired product, including both the cell type and origin of the source material, a detailed nucleotide sequence analysis and a restriction enzyme digestion map of the cloned segment. If a cloned polynucleotide contains more information than the coding sequence, i.e. introns or flanking sequences, then these additional sequences should be identified.

The construction of the vector used for expression of the cloned nucleotide segment into its respective product should also be thoroughly described. This description should include a detailed explanation of the source and function of the component parts of the vector e.g. origins of replication, antibiotic resistance genes, promoters, enhancers, whether or not the product is being synthesized as a fusion protein. The restriction enzyme digestion map of the entire constructed vector should be provided.

The host cell system which will generate the product is coordinated to fit the expression vector. It is, therefore, important that a complete description of the source, relevant phenotype and genotype of the host be provided. If the host cell is of mammalian origin then data concerning its

tumorigenicity should be obtained. Various methods can be utilized to transfer an expression vector into its host, such as transfection, transduction, infection, microinjection, etc. The mechanism of transfer, copy number, and the physical state of the vector inside the host cell, integrated or extrachromosomal, should be provided. In most cases, a single host cell containing the expression vector should be cloned to give rise to the Master Cell Bank. The cloning history and methodology should be described. If new Master Cell Banks are to be generated periodically by expression vector transfer and cloning, acceptance criteria for both the new clones and the product produced by these clones should be described.

IV. Master Cell Bank

Host cells chosen to express recombinant DNA products should be maintained in seed lot aliquots under storage conditions which ensure genetic stability. The stability of both the host cell and expression vector should be investigated. In particular, the fidelity of the nucleotide sequence encoding the expression product present in each seed lot should be verified.

The purity of the cells in the seed lot should be assured by isoenzyme analysis, auxotrophy, antibiotic resistance, and karyology, as appropriate.

The seed lot should be free of adventitious agents including mycoplasma, bacteria, yeast, viruses, and virus-like particles. At a minimum, mammalian host cells should be analyzed for reverse transcriptase and detection of virus-like structures by electron microscopy.

V. Production

The cells used in each production run should be characterized by analysis of relevant phenotypic or genotypic markers, and tested for adventitious agents in samples taken just prior to termination of culture.

The procedures and materials used for cell growth and induction of product expression should be described in detail.

Data on the consistency of yield of the product from full-scale culture should be maintained, and criteria for the rejection of culture lots should be established.

Penicillin and other beta-lactam containing antibiotics should not be used in production runs.

VI. Purification

The methodology of harvesting, extraction, and purification should be described in detail, and the removal of any toxic chemicals introduced by these procedures should be demonstrated.

The extent of purification of recombinant DNA products should be consistent with the intended use of the product. Biologicals which are to be administered repeatedly or at high concentrations should be adequately pure to prevent the development of undesired immune or toxic reactions to contaminants. Although recombinant DNA products may be demonstrated to be 99% pure by physicochemical characterization, special attention should be directed toward the removal of certain contaminants which may be present in small amounts. The purification process should specifically eliminate undesirable antigenic materials and detectable viruses, microbial contamination and nucleic acids.

The use of monoclonal antibodies for affinity purification of recombinant DNA products deserves special comment. A document entitled "Points to Consider in the Manufacture of Monoclonal Antibody Products for Human Use" already has been issued by the National Center for Drugs and Biologics. The monoclonal antibodies should be shown to be free of unwanted biologically active substances such as DNA and viruses as described in Section VII, C. Methods used for the coupling of the monoclonal antibody to the column matrix and the removal of contaminants from the affinity column should be described. The final product should be examined for the presence of antibody and column matrix.

VII. Characterization of the Product

Evidence for purity of the product and for the identity of the product with reference preparations may be derived from the results of a wide variety of tests. Such tests may include:

A. Physicochemical Characterization of Proteins

1. Compositional Analysis by Amino Acid Analyses

The complete amino acid composition of the peptide or protein should include accurate values for methionine, 1/2-cystine and tryptophan, which may require sample preparation procedures other than hydrolysis in 6N HCl. The amino acid composition presented should be the average of at least three (3) separate determinations of the same product or lot number. A positive correlation in the results of analyses of the product and reference preparations would support arguments for identity.

For small proteins or peptides with molecular weight less than 10,000 daltons, the demonstration of nearly integral ratios of amino acids would support arguments of peptide purity.

For proteins with molecular weight in excess of 10,000 daltons, the amino acid compositional analysis may not provide as useful information in support of the purity of the product as for the small proteins or peptides. However, integral values for those amino acid residues generally found in low quantities, such as tryptophan and/or methionine, could be obtained and used to support arguments of purity.

2. Partial Sequence Analysis

Partial amino terminal (15 residues) and carboxy terminal sequence analyses can serve as important criteria for the identity of recombinant DNA produced proteins or peptides. The sequence data presented in tabular form should include the total yield for every amino acid at each cycle as well as the repetitive yield for the major sequence(s). Unexpected heterogeneity in the amino termini and carboxy termini of proteins produced by recombinant DNA technology due to proteolytic degradation has been revealed already by sequence analysis.

3. Peptide Mapping

Peptide mapping can provide a very discriminating comparison between a recombinant DNA product and an authentic sample of the natural product or a reference preparation. This is best accomplished using a pure and specific peptidase to cleave the proteins under identical conditions, followed by either two-dimensional electrophoresis or high performance liquid chromatography. In conjunction with amino acid composition and sequence analysis of each peptide,

peptide mapping can provide precise evidence for the identity of a protein. For proteins containing disulfide bonds, peptide mapping can be used to verify the proper arrangement of disulfide bonds in the final product.

4. Polyacrylamide Gel Electrophoresis (PAGE) and Isoelectric Focusing

PAGE and isoelectric focusing are valuable techniques for verifying the purity and molecular weight of proteins and peptides. The PAGE gels should be run in sodium dodecylsulfate with and without exposure to reducing agents, and with appropriate molecular weight standards or reference preparations.

It is preferable to analyze duplicate samples on slab gels, one stained with Coomassie blue and the other stained with silver. The silver stain is considerably more sensitive for the detection of very small quantities of proteins and is useful in identifying nonprotein materials such as nucleic acid, carbohydrate and lipid which may be present.

For peptides of molecular weight less than ca. 8,000 daltons, most PAGE methods may not be accurate for molecular weight estimates.

5. High Performance Liquid Chromatography (HPLC)

HPLC is a very useful method to determine the purity of a protein or peptide, to evaluate its molecular configuration and, under some circumstances, to confirm its identity. HPLC may be especially useful in characterizing and quantitating specific impurities in the final product. A widely used method is reverse-phase HPLC. A protein or peptide which elutes as a single symmetrical peak in two markedly different systems, including an ion-pair system, is generally quite pure.

6. Circular Dichroism and Optical Rotatory Dispersion (CD and ORD)

Comparison of the CD or the ORD spectrum of the material prepared by recombinant DNA technology with the corresponding spectrum of the native material or the reference preparation may support conformational similarity.

7. Other Characterization

Additional physicochemical characterizations may be appropriate for recombinant DNA products incorporating carbohydrates, DNA, lipids, and other nonprotein components.

B. Biological tests for Identity and Potency

Comparing the recombinant DNA product to the natural product in a suitable bioassay will provide additional evidence relating to the identity and potency of the recombinant DNA product.

Various types of bioassay may be used. In vitro assays are usually faster, less expensive, and more precise than animal studies, yet adequate testing of a biological product before introduction into humans may involve animal studies. Most vaccines produced by recombinant DNA techniques should be compared to the natural substance with respect to their ability to promote immune responses in animals. The extent and frequency of animal testing should be determined on a case-by-case basis.

C. Tests for contaminants

Reliable and sensitive tests will be needed to assay for trace contamination and product related impurities in individual production batches. Although physicochemical characterization can ensure a high degree of purity of a recombinant product, tests for trace contaminants will rely heavily on biological indicator systems.

1. Pyrogen Contamination

Pyrogenicity testing should be conducted by traditional methods of endotoxin detection with limulus amebocyte lysate (LAL) and direct pyrogenicity testing by injection of rabbits with the final product. Criteria comparable to those adopted for acceptance of the natural product should be used for the DNA product.

Certain biological pharmaceuticals may be pyrogenic in humans despite having passed the LAL test and the rabbit pyrogen test. This phenomenon may be due to nonendotoxin contaminants which appear to be pyrogenic only in humans. To attempt to predict whether human subjects will experience a pyrogenic response, human blood mononuclear cells can be cultured in vitro with the final product and the cell culture fluid injected into rabbits. A fever in the rabbits indicates that the product contains pyrogenic substances.

2. Viral Contamination

Tests for viral contamination should be appropriate to the cell substrate and culture conditions employed. Absence of contamination of the final product by adventitious viruses should be demonstrated.

3. Nucleic Acid Contamination

Removal of nucleic acid at each step in the purification process should be demonstrated in pilot experiments by examining the extent of elimination of added radiolabeled host cell DNA. This analysis will provide the theoretical extent of the removal of nucleic acid during purification.

A direct analysis of nucleic acid in the final product should be performed by hybridization analysis of immobilized contaminating nucleic acid utilizing appropriate probes, such as both nick-translated host cell and vector DNA. This method ought to provide sensitivity to the level of 10 picograms per

dose. Theoretical concerns regarding transforming DNA derived from the cell substrate will be minimized by the general reduction of contaminating nucleic acid.

4. Antigen Contamination

Products which are administered repeatedly and/or in large doses should be assayed for trace antigenic constituents likely to contaminate the final product. Tests such as Western blots, radioimmunoassays and enzyme-linked immunosorbant assays using high affinity antibodies raised against host cell lysates, appropriate subcellular fractions or culture medium constituents, should be used to detect contaminating antigens. Such methods can provide sensitivity in the range of 1 to 100 parts per million. Because the detection of antigens will be limited by the specificity and sensitivity of the antisera used, these immunoassays will complement but not replace silver stain analysis of SDS-PAGE gels. Patients given large or repeated doses of a product should be monitored for the production of antibodies to potentially contaminating antigens.

5. Microbial Contamination

Tests for microbial contamination should be conducted using prescribed sterility testing procedures. Absence of bacteria (aerobes and anaerobes), fungi, yeast and mycoplasma in the final product should be demonstrated.

D. Toxicity tests in animals

A recombinant DNA product that is demonstrated to be identical to a natural substance for which the pharmacology and toxicology data are already developed will generally not be required to undergo all the animal toxicity tests normally carried out in the evaluation of a new product. Any recombinant DNA product

consisting of a natural substance with a minor modification would require somewhat more testing in appropriate animal models. A product radically altered from the natural substance would require still more extensive animal tests, including those for carcinogenicity, teratogenicity and effects on fertility. The specific tests which might be appropriate are best addressed on a case-by-case basis with the appropriate Office.

VIII. Modified Protein Products

Using recombinant DNA procedures it may be possible to modify the structure of biological proteins to enhance their desired biological properties and/or diminish undesirable ones. Any substance which is not a natural constituent of the human body may be antigenic and also may cause unknown and possibly adverse biological effects. Whether such a product should be used in humans depends on a careful assessment of its new benefits compared to the risks identifiable during its preclinical and clinical evaluation.

IX. Clinical Trials

Clinical trials will be necessary for all products derived from recombinant DNA technology to evaluate their safety and efficacy.

National Humanities Center
7 Alexander Drive
Research Triangle Park,
N.C. 27709
March 11, 1985

Dear LeRoy:

...I have read with interest the proposed guidelines for gene therapy, and am impressed with the care and precision in the drafting. Time did not permit me to make any comments before the deadline, but I do want to add one or two observations.

First, and most important, I do appreciate the reasons for confining your attention to somatic-cell therapy--just as I appreciated the reasons for confining the attention of the Asilomar conference to questions of biohazard (notwithstanding the presence of Soviet scientists and their known interest in biological weapons). But I am not convinced about the theoretical or practical soundness of the separation. The organism is, after all, a whole, with interacting parts. Just because we choose to make the soma/germ line distinction, for the sake of our purposes, does not mean that these are two non-communicating realms. Somatic cells break down, and their products may find their way into the blood stream. Inflammatory processes in the gonads could bring high concentration of genetically transformed lymphocytes into proximity to gamete producing cells, and the possibility of genetic transformation is, at least in principle, a possibility to be reckoned with and excluded by experiment--not by definition. Mind you, the risk might still be very much worth taking but, as your point II-C-1 indicates but does not stress, it must be considered and identified--and made more than a matter of optional comment, as you now have it. Indeed, the whole of point II-C suggests that at least some members of your panel are not entirely comfortable with the neat separation, and for good reason. The techniques for gene therapy of

specific somatic cells will almost certainly make germ-line genetic manipulation more possible technically (your wording in II-C-3, "will lead to" is hurtfully ambiguous: technical possibility is one thing, likely practice another), and it is somewhat naive, I believe, to treat this as a matter for predictive comment, on an optional basis, only. I would prefer that each applicant be asked to directly respond to the question of whether and how the work he is proposing to do might make more feasible the possibilities listed in II-C-3 (a) & (b). You will not get such information unless you insist on it, and, to my view, it should be treated not as a remote possibility, but as something on which we must be properly informed.

Second, a minor point. Item I-C-1 (the number "1" is missing) talks about "equity consideration." I am afraid that I don't know what you are talking about. The term is too abstract and remote. Do you mean something like: "How will patients fairly be chosen, if there are more who need treatment than can be treated?" A general comment: the ethical points, especially as presented to working scientists, should be presented more expansively and less abstractly--free from the kind of jargon that will produce only jargonized answers.

Third, II-A: Should you not here ask the researchers first to try now to anticipate for you what those public concerns might be-- and not only how they will proceed to provide the public with information? Just as NIH proposals are asked to talk about possible significance of the research, so too you might ask researchers to identify, for the RAC, the possible dangers/risks/public concerns related to the work. The question itself will be useful in the education of the scientist and of the RAC....

With all best wishes,

Yours,

Leon R. Kass

POINTS OF CONSIDER IN THE DESIGN AND SUBMISSION OF HUMAN SOMATIC-CELL GENE THERAPY PROTOCOLS

Introduction

Experiments in which recombinant DNA[1] is introduced into cell of a human subject with the intent of stably modifying the subject's genome are covered by Section III-A-4 of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (49 FR 46266). Section III-A-4 requires such experiments to be reviewed by the NIH Recombinant DNA Advisory Committee (RAC) and approved by the NIH. RAC consideration of each proposal will be on a case-by-case basis and will follow publication of a precis of the proposal in the Federal Register, an opportunity for public comment, and review of the proposal by the working group of the RAC. RAC recommendations on each proposal will be forwarded to the NIH Director for a decision, which will then by published in the Federal Register. In accordance with Section IV-C-1-b of the NIH Guidelines, the NIH Director may approve proposals only if he finds that they present "no significant risk to health or the environment."

In general, it is expected that somatic-cell gene therapy protocols will not present a risk to the environment as the recombinant DNA is expected to be confined to the human subject. Nevertheless, item I-B-4-b of the "Points to Consider" document asks the researchers to address specifically this point.

[1]Experiments using retroviruses (RNA) as vectors are also covered by the NIH Guidelines for Research Involving Recombinant DNA Molecules and hence by this document. Section III-A-4 applies to both recombinant DNA and DNA derived from recombinant DNA.

This document is intended to provide guidance in preparing proposals for NIH consideration under Section III-A-4. Not every point mentioned in the document will necessarily require attention in every proposal. It is expected that the document will be considered for revision at least annually as experience in evaluating proposals accumulates.

A proposal will be considered by the RAC only after the protocol has been approved by the local Institutional Biosafety Committee (IBC) and by the local Institutional Review Board (IRB) in accordance with Department of Health and Human Services regulations for the protection of human subjects (45 CFR, Part 46). If a proposal involves children, special attention should be paid to subpart D of these regulations. The IRB and IBC may, at their discretion, condition their approval on further specific deliberation by the RAC and its working group. Consideration of gene therapy proposals by the RAC may proceed simultaneously with review by any other involved federal agencies (e.g., the Food and Drug Administration) provided that the RAC is notified of the simultaneous review. Meetings of the committee will be open to the public except where trade secrets or proprietary information would be disclosed. The committee would prefer that the first proposals submitted for RAC review contain no proprietary information or trade secrets, enabling all aspects of the review to be open to the public. The public review of these protocols will serve to educate the public not only on the technical aspects of the proposals but also on the meaning and significance of the research.

This document is designed to cover the initial protocols on human gene therapy. It is expected that these first cases will involve only one or a very few patients using biological material prepared under the direct personal supervision

of the principle investigator. If and when gene therapy protocols become more widely applied and use manufactured biological material, then the FDA publication "Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by RDNA Technology," or the appropriate document at that time, should be consulted.

The clinical application of recombinant DNA techniques to human gene therapy raises two general kinds of questions. Part I of this document deals with the short-term risks and benefits of the proposed research to the patient[2] and to other people as well as with issues of equity in the selection of subjects, informed consent, and privacy and confidentiality. In Part II, investigators are requested to address broader ethical and social issues pertaining to the research and its longer-term implications. These broader questions go beyond the usual purview of IRBs and reflect general public concerns about biomedical research. Part III of the "Points to Consider" summarizes other documentation that will assist the RAC and its working group in their review of gene therapy proposals.

The Working Group on Human Gene Therapy has concluded that somatic-cell gene therapy does not differ fundamentally from other accepted practices in medicine, such as organ or bone-marrow transplantation. It bases this conclusion on such important public-policy documents as the Congressional hearing on "Human Genetic Engineering" (1982), the report of a presidential commission entitled Splicing Life (1982), and a recent Office of Technology Assessment background paper entitled Human Gene Therapy (1984). In light of this conclusion, the working

[2]The term "patient" and its variants are used in the text as a shorthand designation for "patient-subject."

group judges that it is appropriate to review somatic-cell gene therapy protocols at this time and to approve those protocols if they adequately address the questions raised in the following document. However, in recognition of the social concern that surrounds the general practice of human gene therapy, the working group will, in its review of individual somatic-cell proposals, also consider the following issues: (1) how strong is the evidence that somatic-cell gene therapy does not affect reproductive cells; (2) does somatic-cell gene therapy make it more likely that recombinant DNA techniques will be employed for: (a) germ-line gene therapy; (b) the enhancement of human capabilities through genetic means; or (c) eugenic programs encouraged or even mandated by governments? If so, what are the implications of proceeding with somatic-cell gene therapy?

Responses to the questions raised in these "Points to Consider" should be in the form of either written answers or references to specific sections of the protocol or other documentation which accompanies the proposal.

I. Description of Proposal

A. Objectives and rationale of the proposed research

State concisely the overall objectives and rationale of the proposed study. Please provide information on the following specific points:

1. Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?

2. Describe the natural history and range of expression of the disease selected for treatment. In your view, are the usual effects of the

disease predictable enough to allow for meaningful assessment of the results of gene therapy?

3. Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

4. What alternative therapies exist? In what groups of patients are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene therapy?

B. Research design, anticipated risks and benefits

1. Structure and characteristics of the biological system

Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

a. What is the structure of the cloned DNA that will be used?

(1) Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete sequence analysis or a detailed restriction map of the total construct.

(2) What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)?

(3) Describe the steps used to derive the DNA construct.

b. What are the structure and composition of the material that will be administered to the patient?

(1) Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells.

(a) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)? What tests have been used and what is the sensitivity of the tests?

(b) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (nucleic acids, proteins, etc.) or contaminating viruses or other organisms in the cells or serum?

(2) Describe any other material to be used in preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

2. Preclinical studies, including risk-assessment studies

Describe the experimental basis (derived from tests in cultured cells and laboratory animals) for claims about the efficacy and safety of the proposed system for gene delivery.

a. Laboratory studies of the delivery system

(1) What cells are the intended recipients of gene therapy? If recipient cells are to be treated *in vitro* and returned to the patient, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the treated cells will act as recipients?

(2) Is the delivery system efficient in the sense that it results in the insertion of the desired unrearranged DNA sequences into the target cells of the patient.

(3) How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated?

(4) How many copies are inserted per cell? How stable is the inserted DNA both in terms of its continued presence and its structural stability?

b. Laboratory studies of gene expression

Is the inserted gene expressed? To what extent is expression only from the desired gene (and not from the surrounding DNA)?

In what percentage of cells does expression occur? Is the product biologically active? What percentage of normal activity results from the inserted gene? Is the gene expressed in cells other than the target cells? If so, to what extent?

c. Laboratory studies pertaining to the safety of the delivery/expression system

(1) If a retroviral system is used:

(a) What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

(b) How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

(c) What laboratory evidence is available concerning potential harmful effects of the treatment, e.g., malignancy, harmful mutations, regeneration of infectious particles, or immune responses? What steps have been taken in

designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

(d) Is there evidence from animal studies that vector DNA has entered untreated cells and specifically germline cells? What is the sensitivity of the analyses?

(e) Has a protocol similar to the one proposed for a clinical trial been carried out in non-human primates and with what results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

(2) If a non-retroviral delivery system is used: What animal studies have been done to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated)? How long have the animals been studied after treatment? What tests have been used and what is their sensitivity?

3. Clinical procedures, including patient monitoring

Describe the treatment that will be administered to patients and the diagnostic methods that will be used to monitor the success or failure of the treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study.

a. Will cells (e.g., bone marrow cells) be removed from patients and treated in vitro in preparation for gene therapy? If so, what kinds of cells will be removed from the patients, how many, how often, and at what intervals?

b. Will patients be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy) prior to gene therapy?

c. What treated cells (or vector/DNA combination) will be given to patients in the attempt to administer gene therapy? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple treatments? If so, over what period of time?

d. What are the clinical endpoints of the study? How will patients be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be done? How long will patient follow-up continue?

e. What are the major potential beneficial and adverse effects of treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of potential adverse effects on patients with the probability and magnitude of deleterious consequences from the disease if gene therapy is not performed.

f. If a treated patient dies, will an autopsy be requested? If so, please indicate what special studies, if any, will be performed.

4. Public health considerations

Describe the potential benefits and hazards of the proposed therapy to persons other than the patients being treated.

a. What potential benefits or hazards are postulated?

b. Is there any expectation that the recombinant DNA will spread from the patient to others or to the environment?

c. What precautions will be taken, if any, to protect others (e.g., patients sharing a room, health-care workers, or family members) from such potential hazards?

5. Qualifications of investigators, adequacy of laboratory and clinical facilities

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of gene therapy. In addition, please describe the laboratory and clinical facilities where the proposed study will be performed.

a. What professional personnel (medical and nonmedical) will be involved in the proposed study? What are their specific qualifications and experience with respect to the disease to be treated and with respect to the techniques employed in molecular biology? Please provide curricula vitae (see III-D).

h. At what hospital or clinic will the treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will patients occupy regular hospital beds or clinical research center beds? Where will patients reside during the follow-up period.

C. Selection of subjects

Estimate the number of patients to be involved in the proposed study of gene therapy. Describe recruitment procedures and patient eligibility requirements paying particular attention to whether these procedures and requirements are fair and equitable.

1. How many patients do you plan to involve in the proposed study?

2. How many eligible patients do you anticipate being able to identify each year?

3. What recruitment procedures do you plan to use?

4. What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

5. How will patients be selected fairly, if it is not possible to treat all who need treatment?

D. Informed consent

Indicate how patients will be informed about the proposed study and how their consent will be solicited. The consent form should adhere to the requirements of 45 CFR 46. If the study involves pediatric or

mentally handicapped patients, describe procedures for seeking the permission of parents or guardians and, where applicable, the assent of each patient. Areas of special concern highlighted below include potential adverse effects, financial costs, privacy, and the right to withdraw from further participation in the study.

1. Will the major points covered in Sections I-A through I-C of this document be disclosed to potential participants in this study and/or parents or guardians in language that is understandable to them? (Include a copy of the patient consent form as part of the documentation requested in Part III below.)

2. Will the innovative character and the theoretically-possible adverse effects of gene therapy be discussed with patients and/or parents or guardians? Will the potential adverse effects be compared with the consequences of the disease? What will be said to convey that some of these adverse effects, if they occur, could be irreversible?

3. Will the financial costs of gene therapy and any available alternative therapies be explained to patients and/or parents or guardians?

4. Will patients and/or their parents or guardians be informed that the innovative character of gene therapy may lead to great interest by the media in the research and in treated patients? What special procedures, if any, will be followed to protect the privacy of patients and their families?

5. Will patients and/or their parents or guardians be informed of their right to withdraw at any time from the proposed study and of the consequences of withdrawal at the various stages of the experiment? State the extent to which subjects will be specifically advised of the reversibility or irreversibility of procedures that are performed during the course of the experiment.

F. Privacy and confidentiality

Indicate what measures will be taken to protect the privacy of gene therapy patients and their families as well as to maintain the confidentiality of research data.

1. What provisions will be made to honor the wishes of individual patients (and the parents or guardians of pediatric or mentally handicapped patients) as to whether, when, or how the identity of patients is publicly disclosed?

2. What provision will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual patients?

II. Social Issues

The following issues are beyond the normal purview of local IRBs. However, since these issues have arisen in public debates about human gene therapy and the potential future applications of genetic techniques, the RAC and its working group request that investigators respond to questions A and B below.

A. What steps will be taken to ensure that accurate information is made available to the public with respect to such public concerns as may arise from the proposed study?

B. Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among investigators and clinicians concerning research methods and results?

III. Requested Documentation

In addition to responses to the questions raised in these "Points to Consider," please submit the following materials:

A. Your protocol (including consent form) as approved by your local IRB and IBC.

B. Local IRB and IBC minutes and recommendations that pertain to your protocol.

C. A one-page abstract of the gene therapy protocol.

D. Curricula vitae for professional personnel.

E. An indication of other federal agencies to which the protocol is being submitted for review.

F. Any other material which you believe will aid in the review.

IV. Reporting Requirements

A. Serious adverse effects of treatment should be reported immediately to both your local IRB and the NIH Office for Protection from Research Risks (phone: 301-496-7005).

B. Reports regarding the general progress of patients should be filed at six-month intervals with both your local IRB and the NIH Office of Recombinant DNA Activities (ORDA) (phone: 301-496-6051). These twice-yearly reports should continue for a sufficient period of time to allow observation of all major effects (at least three to five years). If a patient dies, the autopsy report should be submitted to the IRB and ORDA.

Federal Register

Thursday
March 28, 1985

Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA; Notice of Meeting and Proposed Actions Under Guidelines for Research

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Pub. L. 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee at the National Institutes of Health, Building 31C, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20205, on May 3, 1985, from 9:00 a.m. to adjournment at approximately 5:00 p.m. This meeting will be open to the public to discuss:

Report of the Working Group on Release into the Environment;
Report of the Working Group on Human Gene Therapy;
Proposed coordinated framework for regulation of biotechnology;
Proposed working group on biological weapons;
Amendment of Guidelines; and
Other matters to be considered by the Committee.

Attendance by the public will be limited to space available. Members of the public wishing to speak at the meeting may be given such opportunity at the discretion of the chair.

Dr. William J. Gartland, Jr., Executive Secretary, Recombinant DNA Advisory Committee, National Institutes of Health, Building 31, Room 3B10, telephone (301) 496–6051, will provide materials to be discussed at the meeting, rosters of committee members, substantive program information. A summary of the meeting will be available at a later date.

Dated: March 8, 1985.

Betty J. Beveridge,

Committee Management Officer, NIH.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

[FR Doc. 85–7063 Filed 3–27–85; 8:45 am]

BILLING CODE 4140–01–M

Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, HHS.

ACTION: Notice of Proposed Actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth proposed actions to be taken under the NIH Guidelines for Research Involving Recombinant DNA Molecules. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its meeting on May 3, 1985, the Director of the National Institutes of Health will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments must be received by April 29, 1985.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m. Comments received by close of business April 26, 1985, will be reproduced and distributed to the RAC for consideration at its May 3, 1985, meeting.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from Drs. Stanley Barban and Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205. (301) 496–6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following actions under the Guidelines for Research Involving Recombinant DNA Molecules.

I. Proposed Points to Consider for Environmental Testing of Microorganisms

Deliberate release into the environment of any organism containing recombinant DNA, except certain plants as described in Appendix L, falls under Section III–A of the NIH Guidelines. Experiments in this category cannot be initiated without submission of relevant information on the proposed experiments to NIH, review by the RAC after publication for public comment, and specific approval by NIH.

The RAC Working Group on Release Into the Environment has prepared draft submission guidelines for individuals preparing proposals involving testing in the environment of microoganisms derived by recombinant DNA techniques. The proposed guidance follows:

Points To Consider for Submissions Involving Testing in the Environment of Microorganisms Derived by Recombinant DNA Techniques

Experiments in this category require specific review by the Recombinant DNA Advisory Committee (RAC) and approvals by the National Institutes of Health (NIH) and the Institutional Biosafety Committee (IBC) before initiation. The IBC is expected to make an independent evaluation although this evaluation need not occur before consideration of an experiment by the RAC. Relevant information on the proposed experiments should be submitted to the Office of Recombinant DNA Activities (ORDA). The objective of this review procedure is to evaluate the potential environmental effects of testing of microorganisms that have been modified by recombinant DNA techniques.

These following points to consider have been developed by the RAC Working Group on Release into the Environment as a suggested list for scientists preparing proposals on environmental testing of microorganisms, including viruses, that have been modified using recombinant DNA techniques. The review of proposals for environmental testing of modified organisms is being done on a case-by-case basis because the range of possible organisms, applications, and environments indicate that no standard set of procedures is likely to be appropriate in all circumstances. However, some common considerations allow the construction of points to consider such as those below. *Information on all these points will not be necessary in all cases but will*

depend on the properties of the parental organism and the effect of the modification on these properties.

Approval of small-scale field tests will depend upon the results of laboratory and greenhouse testing of the properties of the modified organism. We anticipate that monitoring of small-scale field tests will provide data on environmental effects of the modified organism. Such data may be a necessary part of the consideration of requests for approval of large-scale tests and commercial applications.

I. Summary

Present a summary of the proposed trial including objectives, significance, and justification for the request.

II. Genetic Considerations of Modified Organism to be Tested

A. Characteristics of the Nonmodified Parental Organism

1. Information on identification, taxonomy, source, and strain.

2. Information on organism's reproductive cycle and capacity for genetic transfer.

B. Molecular Biology of the Modified Organism

1. Introduced Genes

a. Source and function of the DNA sequence used to modify the organism to be tested in the environment.

B. Identification, taxonomy, source, and strain or organism donating the DNA.

2. Construction of the Modified Organism

a. Describe the method(s) by which the vector with insert(s) has been constructed. Include diagrams as appropriate.

b. Describe the method of introduction of the vector carrying the insert into the organism to be modified and the procedure for selection of the modified organism.

c. Specify the amount and nature of any vector and/or donor DNA remaining in the modified organism.

d. Give the laboratory containment conditions specified by the NIH Guidelines for the modified organism.

3. Genetic Stability and Expression

Present results and interpretation of preliminary tests designed to measure genetic stability and expression of the introduced DNA in the modified organism.

III. Environmental Considerations

The intent of gathering ecological information is to assess to the effects of survival, reproduction, and/or dispersal of the modified organism. For this purpose, information should be provided where possible and appropriate on: (i) Relevant ecological characteristics of the nonmodified organism; (ii) the corresponding characteristics of the modified organism; and (iii) the physiological and ecological role of donated genetic sequences in the donor and in the modified organism(s). For the following points, provide information where possible and appropriate on the nonmodified organism and a prediction of any change that may be elicited by the modification.

A. Habitat and Geographic Distribution

B. Physical and Chemical Factors Which Can Affect Survival, Reproduction, and Dispersal C. Biological Interactions

1. Host range.

2. Interactions with and effects on other organisms in the environment including effects on competitors, prey, hosts, symbionts, predators, parasites, and pathogens.

3. Pathogenicity, infectivity, toxicity, virulence, or as a carrier (vector) of pathogens.

4. Involvement in biogeochemical or in biological cycling processes (e.g., mineral cycling, cellulose and lignin degradation, nitrogen fixation, pesticide degradation).

5. Frequency with which populations undergo shifts in important ecological characteristics such as those listed in III–C points 1 through 4 above.

6. Likelihood of exchange of genetic information between the modified organism and other organisms in nature.

IV. Proposed Field Trials

A. Pre-Field Trial Considerations

Provide data related to any anticipated effects of the modified microorganism on target and nontarget organisms from microcosm, greenhouse, and/or growth chamber experiments that simulate trial conditions. The methods of detection and sensitivity of sampling techniques and periodicity of sampling should be indicated. These studies should include, where relevant, assessment of the following items:

1. Survival of the modified organism.

2. Replication of the modified organism.

3. Dissemination of the modified organism by wind, water, soil, mobile organisms, and other means.

B. Conditions of the Trial

Describe the trail involving release of the modified organism into the environment:

1. Numbers of organisms and methods of application.

2. Provide information including diagrams of the experimental location and the immediate surroundings. Describe characteristics of the site that would influence containment or dispersal.

3. If the modified organism has a target organism, provide the following:

a. Identification and taxonomy.

b. The anticipated mechanism and result of the interaction between the released microorganism and the target organism.

C. Containment

Indicate containment procedures in the event of accidental release as well as intentional release and procedures for emergency termination of the experiment. Specify access and security measures for the area(s) in which the tests will be performed.

D. Monitoring

Describe monitoring procedures and their limits of detection for survival, dissemination, and nontarget interactions of the modified microorganism. Include periodicity of sampling and rationale for monitoring procedures. Collect data to compare the modified organisms with the nonmodified microorganism most similar to the modified organism at the site of the trial. Results of monitoring should be submitted to the RAC according to a schedule specified at the time of approval.

V. Risk Analysis

Results of testing in artificial contained environments together with careful consideration of the genetics, biology, and ecology of the nonmodified and the modified organisms will enable a reasonable prediction of whether or not significant risk of environmental damage will result from the release of the modified organism in the small-scale field test. In this section, the information requested in Sections II, III, and IV should be summarized to present an analysis of possible risks to the environment in the test as it is proposed. The issues addressed might include but not be limited to the following items:

A. The Nature of the Organism

1. The role of the nonmodified organism in the environment of the test site, including any adverse effects on other organisms.

2. Evaluation of whether or not the specific genetic modification (e.g., deletion, insertion, modification of

specific DNA sequences) would alter the potential for significant adverse effects.

3. Evaluation of results of tests conducted in contained environments to predict the ecological behavior of the modified organism relative to that of its nonmodified parent.

B. The Nature of the Test

Discuss the following specific features of the experiment that are designed to minimize potential adverse effects of the modified organism:

1. Test site location and area.
2. Introduction protocols.
3. Numbers of organisms and their expected reproductive capacity.
4. Emergency procedures for aborting the experiment.
5. Procedures conducted at the termination of the experiment.

II. Proposed Revision of Appendix C

Dr. Jack J. Manis of the Upjohn Company, Kalamazoo, Michigan, has proposed that the following kinds of experiments be made exempt under Section III–D–5 and the following language be included in Appendix C of the NIH Guidelines:

Experiments and processes utilizing recombinant DNA containing derivatives of *Streptomyces fradiae* or *Streptomyces lincolnensis* are exempt from the Guidelines at all levels of volume scale when the recombinant DNA molecules contained in these hosts are derived solely from nonpathogenic streptomycetes. The nonpathogenicities of the recombinant DNA sources are determined by the local IBC.

For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

For large-scale fermentation experiments BL1–LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system some latitude in the application of BL1–LS requirements as outlined in Appendix K–II–A through K–II–F is permitted.

Exceptions. Experiments described in Section III–A which require specific RAC review and NIH approval before initiation of the experiment.

Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval (see Section III–B–5).

Explanation of this proposed modification is provided in the submission.

III. Proposed Amendment of Part III

In a memorandum dated February 12, 1985, Dr. Bernard Talbot, Deputy Director of the National Institute of Allergy and Infectious Diseases, noted that under the NIH Guidelines for Research Involving Recombinant DNA Molecules certain proposals are received by NIH for review by the NIH Recombinant DNA Advisory Committee (RAC) and subsequent NIH approval. These include proposals which are required to be submitted from institutions that receive support for recombinant DNA research from NIH, and also proposals voluntarily submitted by institutions that receive no NIH support for recombinant DNA research. Recently other Federal agencies have made steps toward assuming new roles in review of recombinant DNA proposals.

Because of these developments, it could now happen that a proposal submitted to the NIH for RAC review and NIH approval (either from an institution that receives NIH funding for recombinant DNA research or voluntarily submitted by an institution that receives no such support) may be also submitted to another Federal agency for review.

Dr. Talbot states:

In such a case, I believe it would be advantageous for NIH to have the option of deferring to the review and approval by the other Federal agency rather than always going through review and approval by both the other Federal agency and the NIH. In order to allow this, I request that the following proposed change in the NIH Guidelines be issued for public comment, and placed on the agenda of the next RAC meeting. I propose that a new sentence be added at the end of Section III–A of the Guidelines ("Experiments that Require RAC Review and NIH and IBC Approval Before Initiation") just before Section III–A–1 of the Guidelines, as follows: "If experiments in this category are submitted for review to another Federal agency, the submitter shall notify ORDA; ORDA may then determine that such review serves the same purpose, and based on that determination, notify the submitter that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal Agency."

Additional background information is provided in the memorandum.

IV. Proposed RAC Working Group

Messrs. Lee Rogers and Jeremy Rifkin of the Foundation on Economic Trends, Washington, D.C., submitted the following letter dated February 28, 1985, to NIH:

We are formally proposing that the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH) establish a working subgroup whose stated purpose would be to examine potential uses of recombinant DNA technology for offensive and defensive biological weapons systems. In addition, this subgroup will also explore current Department of Defense (DOD) programs specifically designed to develop "defensive" preparedness against the threat of genetic engineering warfare by aggressor nations or terrorists. It should be made clear that such a study is designed to look into the potential as well as actual uses of recombinant DNA technology for military purposes regardless of whether such experimentation is being conducted at this time. The working subgroup on biological warfare will make its findings available to the RAC, NIH, and interested members of the public. The working subgroup may also wish to make recommendations regarding future oversight of recombinant DNA work in this field.

It is no longer possible to ignore the potential military uses of recombinant DNA experimentation in light of the DOD's plan to construct an aerosol test laboratory at Dugway Proving Ground in Utah. The military has stated its intention to use this lab to test defenses against biological warfare experiments and it further stated that it will be working with deadly biological pathogens. In November 1984, the Secretary of Defense, Caspar Weinberger, stated in a letter to Senator Jim Sasser: "We continue to obtain new evidence that the Soviet Union has maintained its offensive biological warfare program and that it is exploring genetic engineering to expand their program's scope. Consequently, it is essential and urgent that we develop and field adequate biological and toxin protection." (See enclosed document.) In light of these recent developments, it is imperative that the RAC begin a thorough and comprehensive study of the potential uses of recombinant DNA technology for military purposes.

Since its inception, RAC has involved itself in every aspect of recombinant DNA technology in an effort to develop procedures, guidelines, protocols, and ethical standards to oversee this research. The only area of recombinant DNA experimentation that has not yet been rigorously examined is the potential military uses. Therefore, I would think that this committee would find it helpful to explore the potential military uses of recombinant DNA technology in order to facilitate a better understanding of the various issues involved. Moreover, it is altogether appropriate for the RAC to engage in such a study as the DOD has stated on many occasions that it is adhering to the guidelines established by this committee and the NIH. An independent study by the RAC of the military potential or recombinant DNA technology can only serve to better inform the Executive Branch, Congress and the public of the issues involved in this particular field.

Dated: March 11, 1985.

Anthony S. Fauci,

Director, National Institute of Allergy and Infectious Diseases, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance.* Normally NIH lists in its announcements the number and title of affected individual programs for the

guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE
WORKING GROUP ON HUMAN GENE THERAPY
MINUTES OF MEETING[1]

APRIL 1, 1985

The Working Group on Human Gene Therapy of the Recombinant DNA Advisory Committe was convened at 9:00 a.m. on April 1, 1985, at the National Institutes of Healt Building 31C, Conference Room 9, 9000 Rockville Pike, Bethesda, Maryland 20205. The meeting was open to the public. Dr. LeRoy Walters was the Chair. The follo ing people were present for all or part of the meeting:

Working Group Members:

W. French Anderson
Judith Areen
Alexander Capron
James Childress
Samuel Gorovitz
Susan Gottesman
Clifford Grobstein
Maurice Mahoney
Robert Mitchell
Arno Motulsky
Robert Rich
LeRoy Walters
Anne Witherby
William Gartland
(Executive Secretary)

A working group roster is attached (Attachment I).

Consultant:

Howard Temin, University of Wisconsin

Liaison Representatives:

Charles McCarthy, National Institutes of Health
Bonnie Lee, Food and Drug Administration

Other National Institutes of Health Staff:

Mary Carter, NINCDS
Elizabeth Milewski, NIAID

[1]The working group is advisory to the RAC, and its recommendations should not be considered as final or accepted.

Others:

Mary Ann Danello, Food and Drug Administration
Luther Val Giddings, Office of Technology Assessment, U.S. Congress
Kathleen Kaslow, Georgetown Medical School
Rihito Kimura, Kennedy Institute of Ethics
Ainer Moufang, Weadon, Dibble, and Rehm
Greta Rosenzweig, NOVA, Public Broadcasting System
Hans-Martin Sass, Georgetown University
Harold Schmeck, New York Times
Bill Walsh, Department of State
Patricia Williams, FDC Reports, Inc.

Dr. Walters called the meeting of the Working Group on Human Gene Therapy to order at 9:05 a.m. on April 1, 1985. He introduced Dr. Temin, the virology consultant, to the working group.

Dr. Walters said the purpose of the meeting was to continue to develop the document "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols." He said the document had appeared in the January 22, 1985, Federal Register (Attachment II) and had been widely distributed for comments. Some comments on this announcement had been received (Attachment III) and a subworking group addressed these comments at a meeting on March 15, 1985. The document before the working group was the result of that subworking group meeting (Attachment IV).

Dr. Walters noted that Dr. Grobstein (Attachment V) had provided alternative versions of several sections of the points to consider document. Dr. Grobstein had drafted alternative language for a paragraph of the introduction and for Section I-B-4, Public health considerations.

Dr. Motulsky suggested the working group begin by discussing in general terms the issues which run though a number of the comments. The working group agreed to this approach.

Dr. Childress said many of the comments had addressed social issues; the subworking group had determined that several of the questions in Part II of the document should be moved to the introduction of the document. With these issues now in the introduction, the working group can indicate it will consider these types of issues, but investigators will not be personally required to address these issues.

Dr. Grobstein felt investigators should not be required to address these issues generically but should be asked to address them for the specific protocol. He indicated that his proposed substitute language rephrased these issues along these lines (Attachment V).

Dr. Gorovitz said the points to consider document now implicitly indicates the working group will address these issues. He agreed that investigators might, however, have specific information to offer concerning their proposals.

Dr. Rich thought asking investigators to address these issues indicates the working group is seriously considering these issues.

Dr. Gottesman did not feel the points to consider document should require investigators to address social issues; rather the working group should address these issues.

Mr. Capron also felt the working group should not require investigators to address social issues. The document might explicitly state these issues are important considerations, but investigators will not be required specifically to address these issues.

Dr. Motulsky said several individuals had questioned the balance and composition of the working group. He noted that some respondants had called for an increase in technical specialists while others had called for an increase in public members. The subworking group at the March 1, 1985, meeting felt the working group as currently constituted was probably at maximal operating size; and the addition of new members would probably decrease working group efficiency.

Dr. McCarthy said the working group as it is currently constituted is the optimal size to perform its function; however, issues requiring special expertise may arise with particular proposals. He suggested the points to consider document should state clearly that additional scientific expertise will be sought during protocol review.

Dr. Grobstein thought the working group as currently constituted is reasonably balanced between technical and social science expertise. He agreed the points to consider document should explicitly state the working group will probably need, however, additional expertise in specific topic areas during particular reviews.

Dr. Bonnie Lee of the Food and Drug Administration (FDA) said the points to consider should more explicity state the purpose of the document and the review responsibilities of the working group and the NIH. Will review focus primarily on the science?

Dr. Gottesman felt the working group may have to split into two subworking groups during review of proposals. One subworking group would address scientific and technical issues; the other subworking group would address social issues. Both groups would then meet in plenary session to discuss the whole proposal.

Dr. Motulsky said the working group should first distinguish and discuss the technical issues; the social issues will then be couched in this technical understanding.

Mr. Capron noted that one commentor (Attachment III) had requested a scientific conference on the use of retroviral vectors. He questioned whether such a conference was necessary.

Dr. Anderson said he had been discussing with representatives of the National Academy of Sciences (NAS) the possibility of NAS sponsoring a public scientific meeting on retroviruses.

Dr. Temin said retroviruses are a complex and highly interesting area of research. He pointed out scientific meetings are usually devoted to the latest and the newest in the field. He questioned whether with such a meeting would reassure the public about the very complicated field of retrovirology. A meeting specifically directed towards public education should have a different format than the proposed NAS meeting.

Dr. Grobstein thought an NAS meeting devoted to retrovirus vectors and their safety would be useful. However, the working group and RAC will be reviewing specific proposals; and the NAS conference probably will not contribute to review of specific proposals.

Dr. Motulsky suggested the working group might organize a meeting of a small group of experts to address retrovirology and retroviral vectors in human gene therapy.

Dr. Gottesman said she would prefer a small meeting of experts who might specifically discuss the use of retrovirus vectors in human gene therapy and educate the scientific community in retrovirology. She said she would like to have an opportunity to discuss the issues with retrovirus experts and determine the parameters of consideration.

Dr. Rich felt the working group had too greatly limited the attention focused on social concerns. He suggested that if the working group sponsors a session on technical issues, it might also consider organizing a session on social and ethical issues. He thought scholarly papers in these topics would be very useful.

Dr. Gorovitz said Mr. Jeremy Rifkin of the Foundation on Economic Trends had referred to the points to consider as regulations (Attachment III); this indicates misunderstanding about the document's purpose. Dr. Gorovitz said the points to consider are simply informational requests to investigators preparing proposals for submission to the NIH for review. He questioned whether the working group should be more explicit in stating that the points to consider are requests for information. Should the working group consider the need for setting standards of review?

Dr. Gorovitz said two points of view exist as to how and when to develop guidelines for review of human gene therapy proposals. One view is that no protocols should be approved before there is a public consensus on human gene therapy. The other view is that such guidelines cannot be developed a benetio but must arise out of experience. He said he held the later view. He wondered whether the working group should explicitly state that guidelines cannot be developed as an independent exercise.

Mr. Capron said a public consensus exists on the appropriateness of applying human somatic cell gene therapy. Many public hearings have explored this topic. Witnesses from every area of the spectrum have supported application of human somatic cell gene therapy, and many reports have repeatedly stated this consensus.

Mr. Capron said the public should be aware the working group feels a consensus exists on the appropriateness of applying human somatic cell gene therapy. The working group is approaching the completion of the points to consider document; and if some individuals are concerned, now is the time for these individuals to make these concerns heard.

Dr. Walters noted that most public comments on reports such as the Office of Technology Assessment (OTA) report "Human Gene Therapy" have been from the parents of ill children.

Mr. Capron, who had been the Executive Director of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research noted that the letter from the Committee for Responsible Genetics (Attachment III) misquotes the President's Commission report "Splicing Life."

Dr. Gorovitz questioned whether the points to consider should explicitly state a consensus exists. Dr. Walters said the introduction tends in that direction.

Dr. Rich suggested although one could interpret the few comments (Attachment III) received on the January 22, 1985, Federal Register as reflecting a broad level of public understanding, this may not be so. He thought the working group should attempt to increase public understanding.

Ms. Witherby supported Dr. Rich's suggestion. She felt the public was not well informed about issues in human gene therapy. The working group should especially attempt to educate the public on the differences between somatic and germ line human gene therapies.

Dr. Gorovitz felt the working group serves two functions; one function is to serve as a filter to review protocols involving human gene therapy, the second function is to provide the public with confidence in the filtering process. These two functions are not mutually exclusive.

Dr. Gottesman said the working group is actually discussing two different topics. The first topic is whether a consensus exists concerning application of somatic cell human gene therapy. The other topic is whether the public is aware of the issues involved with the application of human gene therapy. She said there have been ample public hearings on somatic cell human gene therapy; perhaps the points to consider document should acknowledge these hearings and reports. Public education is an ongoing process, however. The working group might also prepare an educational document, in this way the members of the working group would also educate each other.

Dr. Walters called the attention of the working group to the March 22, 1985, draft version of the points to consider document (Attachment IV). He asked the working group to consider the revised version point by point.

Dr. Temin said the footnote to the introduction is not accurate. That footnote read as follows:

> "Experiments using retroviruses (RNA) as vectors are also covered by the NIH Guidelines for Research Involving Recombinant DNA Molecules and hence by this document. Section III-A-4 applies to both recombinant DNA and DNA derived from recombinant DNA."

Dr. Temin said the vectors to be used in human gene therapy are RNA viruses, and the vectors introduced into the patients cells will be RNA rather than DNA.

Dr. Gottesman said the working group might have to request a modification in the NIH Guidelines to explicitly cover retroviruses. The interpretation of the working group, however, is that recombinant RNA is covered by the NIH Guidelines. The working group agreed the second sentence of the footnote should read: "Section III-A-4 applies to recombinant DNA, as well as to RNA and DNA derived from recombinant DNA."

Dr. Walters said the language in the introduction indicating the working group would prefer the initial proposals submitted for RAC review contain no proprietary information had been modified.

Dr. Motulsky said the points to consider document should indicate that while the working group prefers to review proposals in open session, submission of confidential information is not precluded.

Mr. Capron volunteered to draft language to that effect for this section.

Dr. Grobstein said the suggestion in the points to consider document that the working group will "educate" the public is arrogant; he suggested the word "inform" be substituted for the word "educate."

Dr. Walters called the attention of the working group to new language indicating that when gene therapy becomes widely applied FDA publications should be consulted. Drs. Grobstein, Rich, and Motulsky suggested the term "gene therapy" should be modified by the words "somatic-cell."

Dr. Walters called the attention of the working group to language explaining that the working group has concluded somatic-cell gene therapy does not differ fundamentally from other accepted practices in medicine.

Dr. Grobstein said he had written alternative language (Attachment V) for this section of the introduction since he was concerned with the term "fundamentally."

Dr. Gorovitz asked whether any of the concerns mentioned in the letter (Attachment VI) from Dr. Leon Kass of the National Humanities Center in Research Triangle, North Carolina, were valid. Dr. Motulsky said the argument advanced concerning inflammatory processes of the gonads and high concentrations of genetically modified lymphocytes is not credible.

Dr. Gottesman suggested Dr. Kass was thinking of possible gametic cell transformation through products of somatic cell breakdown in vivo. Dr. Grobstein said this cell transformation argument is very highly speculative; no evidence exists at this time to suggest such transformation occurs in the body. Dr. Temin said if this type of event were occurring in the mammalian body, genes would be constantly inserting at random points in the genome. This does not occur.

Dr. Motulsky said patients receiving this therapy will be very seriously ill individuals. Such highly speculative arguments cannot be seriously considered when weighing the benefits of treating such individuals.

Mr. Capron suggested the points to consider document should describe the types of concerns which have been raised, a description of the process by which these concerns have been discussed, and an explanation of the working group's position vis-a-vis these concerns.

Dr. Mahoney agreed the working group document could explicitly describe the difference between somatic cell gene therapy and germ line gene therapy. The document could also discuss vector characteristics and the probability of the crippled retroviral vector being transmitted to other individuals.

Dr. Temin said the points to consider document insists the vector be an inactivated virus. The use of retroviruses as vectors in human gene therapy is thus a genetic engineering problem; a great deal is known about modifying the retrovirus to inactivate it and the points to consider document reflects this level of knowledge. He explained that when a retrovirus enters a cell, a DNA copy is made from the RNA template. The DNA copy then goes into the chromosomal DNA. This form of the virus is called a provirus. The provirus gets out of the chromosome by copying itself into RNA. Technically, this last step can be prevented by constructing the virus vector in a certain way. Dr. Temin added that 10% of the human genome consists of proviruses; these proviruses are in the germ line cells as well as in other cells in the body.

Dr. Temin said in addition to requiring the investigator to use a crippled vector, the working group could also specify the initial studies should involve transformation of marrow cells by the vector. Since these cells would be transformed outside of the patient's body, they could be examined to determine the point of vector insertion in the genome. The cells the patient receives would then be much better characterized. This procedure would also ensure that the vector has no opportunity to insert in germ line cells.

Dr. Motulsky said he might accept the risk involved with introducing an intact virus vector into a very ill child who could not be helped by other therapies.

Dr. Temin said the working group would have to evaluate different considerations if an intact virus vector were used. For example, the possibility a new virus could be created by recombination would have to be considered.

Mr. Capron agreed systemic introduction of vectors would clearly raise different considerations than the in vitro modification of bone marrow cells. He suggested the points to consider document might specifically state this distinction and request investigators to address these issues. Dr. Temin said the points are currently constructed to elicit this type of information from investigators.

The working group returned to the discussion of Dr. Grobstein's proposed alternate language (Attachment V). Dr. Motulsky said the primary aim of all therapies including human gene therapy is to help the patient. He suggested the words "principally" or "fundamentally" be substituted for the words "in clinical terms."

Mr. Capron and Dr. Grobstein did not agree with this proposal; they felt the words "principally" and "fundamentally" suggest the therapy can differ to some degree from traditional therapies.

Dr. Gottesman said the purpose of human gene therapy does not differ from the purposes of other therapies, and the consequences of human gene therapy need not differ if it is applied correctly. She suggested the words "in terms of consequences" be substituted for the words "in clinical terms." The working group accepted Dr. Gottesman's proposed language. Mr. Capron volunteered to attempt to draft langauge for this section of the document.

Dr. Rich suggested the document should include a statement on the distinction between vertical and horizontal transmission of genes.

Dr. Rich said the working group apparently wishes to construct two different documents. The first document would explain human gene therapy in nontechnical terms and communicate to the public that the working group is considering these issues. The other document would be a technical document specifying the information the working group would like to review. Dr. Rich said the working group needs both documents; he suggested two documents be drafted and appended one to the other.

Dr. Grobstein also questioned whether the working group could deal with both of these issues simultaneously in the same document. Dr. Walters said the points to consider document is directed to two different audiences, the public and the investigator. He felt the introduction to the document could be directed to both of these audiences.

Ms. Witherby supported Dr. Rich's proposal to prepare two documents. She felt all the social issues will come to life when the first protocol is submitted for review; the working group should have at that time a document to explain gene therapy in nontechnical terms and discuss the social issues.

Dr. Anderson said the working group should cite the history of introducing genetic material into humans. The first successful bone marrow transplant was performed in 1968. A young male patient was treated for ADA deficiency; his T-cells were completely replaced by T-cells from a female donor. No one questions the male identity of this patient even though he carries genes from the female donor. Dr. Anderson said the changes which would be introduced into the patient through human gene therapy are smaller than the addition of genetic material which occurs with bone marrow transplants.

Dr. Grobstein said bone marrow transplants and human gene therapy replacement of a defective gene are not fundamentally different yet the public may perceive these two procedures differently. The working group should be prepared to address this difference in perception.

Dr. Walters again drew the attention of the working group to Dr. Grobstein's proposed draft language (Attachment V).

Dr. Temin said question number two in Dr. Grobstein's proposed language (Attachment V) is not a social issue. That question asks how good is the evidence that the proposed somatic cell gene therapy will not inadvertently affect reproductive cells.

Dr. Gorovitz said he was uncomfortable with the language stating the working group judges it appropriate "to approve these protocols if they satisfactorily address the questions raised" in the document. He thought including such language might make it impossible to disapprove of protocols if the investigator simply provides adequate answers to these questions. He felt the working group should not use language which would commit it to a specific position. Mr. Capron agreed; he suggested the working group modify Dr. Grobstein's proposed language.

Dr. Grobstein said the points to consider document could indicate the working group will consider a number of issues in reviewing protocols but not indicate the terms of review. Certain issues could, however, be highlighted in the document.

Dr. Gottesman said the working group might indicate there are no wrong or right answers for some of these questions, but these issues will be considered during protocol review.

Dr. Temin called the attention of the working group to the third question in Dr. Grobstein's proposed language which reads:

> "Does the proposed somatic cell gene therapy contribute toward: (a) intentional germ line gene therapy; (b) the enhancement of, rather than the correction of defects in, human capabilities through genetic means; or (c) governmental or other programs with eugenic objectives?"

Dr. Temin said the answer to all these questions is yes; the knowledge gained through somatic cell human gene therapy will contribute to the information base necessary for intentional germ line therapy. Since the answers are yes, he questioned the rationale for including these issues in the form of questions in the points to consider document.

Mr. Capron said the working group might indicate its attitude towards such applications. A serious study on social control of these processes might be necessary; everything that can be done need not always be done.

Mr. Capron pointed out that research in other scientific areas will also provide information which will increase the capability of implementing human germ line gene therapy. He felt the working group might also address this issue.

Dr. Grobstein thought research with mice and cattle is moving society much more rapidly towards possible human germ line gene therapy than would human somatic cell gene therapy. Dr. Temin said application of somatic cell gene therapy would, however, permit society to begin thinking in terms of genetically modifying humans.

Dr. Walters felt the working group's mandate is to review somatic cell human gene therapy on a case-by-case basis. Discussing issues such as the impact of research on the capability of implementing human germ line gene therapy may exceed the working group mandate.

Mr. Capron said human somatic cell therapy and animal research moves society towards the possibility of human germ line gene therapy; some group should address this issue. The Working Group on Human Gene Therapy should at the minimum recognize the existence of this issue.

Dr. Gorovitz said the working group cannot skirt these issues by falling back on the mandate's limitations. The working group will one day receive a proposal which will bring these issues to life.

Mr. Mitchell said as Chair of the Recombinant DNA Advisory Committee (RAC), he would at this time rule out of order a proposal involving germ line modification; the RAC does not currently have guidelines for reviewing human germ line gene therapy proposals.

Mr. Mitchell said the human gene therapy situation is evolving rapidly; as the working group learns more, attitudes will change. He felt it was important for the working group to focus on reviewing the initial proposals and not become so bogged down in potential ramifications that the goal is lost.

Dr. Gorovitz felt comfortable with the working group reviewing initial protocols even though the working group had not yet discussed whether there are "right" answers to some questions posed in the points to consider document. He asked whether the group agrees proposals should be reviewed.

Dr. Motulsky said the working group should proceed to reviewing proposals. No public body can state that certain areas of research cannot be explored, it is not possible to stop the exploring human mind.

Dr. McCarthy suggested the points to consider could state the working group's purpose and indicate the group would exercise vigilance in overseeing human gene therapy.

Dr. Gottesman summarized the points which had been made concerning the social issues associated with human somatic cell gene therapy. She said the first question is whether the investigator should be required to address these social issues as part of the submission. The second question is whether the working group will address these issues. She said the working group should determine whether it will approve of protocols in the absence of a discussion of social issues. The third point is whether the working group should attempt to address these issues before the first protocols are submitted.

Dr. Childress thought the points to consider document should state in a positive way that investigators will not be required to specifically address social issues associated with human somatic cell gene therapy. The document should also explicitly state the limitations imposed by the working group; e.g., the vector must be constructed to prevent transmission, and germ line human gene therapy protocols will not be reviewed under these points to consider.

Mr. Capron said these social issues cannot be addressed individually; rather they should be addressed generically as parts of a whole. He cited three points which should be evaluated at the same time: (1) information generated

using animal systems will contribute to developing human germ line gene therapy techniques; (2) techniques are not yet sufficiently developed to implement germ line therapy in humans; and (3) society should consider mechanisms to control the way in which germ line therapies are implemented. He said he is not convinced at this time that human germ line therapy would per se present ethical problems.

Dr. Childress said social forces will determine the effect of somatic cell therapy protocols on germ line therapy. Society will not stop the accumulation of knowledge but can attempt to control application of that knowledge.

Dr. Gottesman said the issue is how to deal with the consequences of increased knowledge. The language in the points to consider document could be rephrased to emphasize this issue. Mr. Capron said this language should be carefully drafted and should simply indicate it is worthwhile to evaluate these issues.

Dr. Walters thought the document's introduction might include a statement of the working group's current thinking. He asked Drs. Gottesman and Childress if they would be willing to attempt to redraft language for this section of the document over the lunch hour. Dr. Childress asked if Mr. Capron could assist in drafting this portion of the document. The working group then adjourned for lunch.

Following lunch, Dr. Walters called the attention of the working group to Section I-A, Objectives and rationale of the proposed research, of the document (Attachment IV); he said no changes had been made in this section of the document.

Dr. Walters said one change had been made in Section I-B, Research design, anticipated risks and benefits; Section I-B-1-b had been modified to read: "What are the structure and composition of the material that will be administered to the patient?"

Dr. Temin asked why the word "composition" had been added to this section of the document. The pertinent question is what is the structure; the concept of composition is included in the concept of structure.

Dr. Anderson said the addition of the word "composition" was an attempt to be responsive to the comments of Dr. Henry Miller of the FDA.

Dr. Motulsky thought the points to consider document should be clear and concise; he did not think it reasonable to muddy the document in an attempt to meet political considerations. He suggested the working group delete the word "composition" from Section I-B-1-b. The working group agreed.

Dr. Grobstein asked whether the term "composition" might be relevant in Section I-B-1-b-(1) of the document which requests information on materials which might be administered to the patient. The working group agreed the word "composition" should remain in Section I-B-1-b-(1).

Dr. Walters called the attention of the working group to Section I-B-1-b-(1)-(b) to the document. He noted that the words "nucleic acids" had been substituted

for the acronym "DNA." The working group agreed this was a reasonable modification of the language of this section.

Dr. Walters then called the attention of the working group to Section I-B-2-a-(2). This section requests information on the efficiency of vector insertion in target cells.

Dr. Temin said this language as written addresses efficiency in a narrow sense. He said this section should ask: how many cells have taken up and integrated the vector DNA; how many of these cells have DNA rearrangements; and how many of these cells are expressing the insert DNA.

Dr. Walters asked if Drs. Temin and Anderson would compose language for this section of the document.

Dr. Walters called the attention of the working group to Section I-B-2-c-(1) of the document.

Dr. Grobstein thought the word "particularly" should be substituted for the word "specifically" in Section I-B-2-c-(1)-(d). The working group agreed.

Dr. Motulsky noted that some comments had been received concerning Section I-B-2-c-(1)-(e) of the document; in response to these comments, he suggested the working group delete the phrase "non-human primates" and substitute the word "animal."

Dr. Anderson said valid reasons exist for requiring primate testing; i.e., the endogenous retroviruses present in other animals differ from those present in primates. He argued that this language should not be deleted from the points to consider document. Dr. Temin said the requirement for primate testing is an important one; primates are the best model system.

Dr. Motulsky asked how long the tested primates will be followed. Dr. Anderson said the primates need only be observed for acute effects. He said some research centers are testing animal models other than primates. He felt data generated by these test systems supplemented with other appropriate data would be adequate.

Dr. Grobstein felt deleting at this time the language requiring primate testing could stir controversy. He thought this language should remain in the document since it does not require such testing be part of the submission, but simply asks that such data be supplied if it exists. Dr. Anderson thought the document indicates primate testing data should be submitted, or a reasonable justification should be given as to why such data were not included. The need to treat a very sick child is a valid justification.

Dr. Temin suggested the working group could add the words "and/or other animals" to Section I-B-2-c-(1)-(e). Dr. Anderson supported this suggestion.

Dr. Motulsky suggested the language should read "animals including nonhuman primates." Dr. Temin thought the language proposed by Dr. Motulsky more strongly specified testing in primates. Dr. Anderson preferred the language

be as neutral as possible; when the data are submitted, the working group could decide if adequate information had been supplied.

The working group agreed to add the phrase "and/or other animals" to Section I-B-2-c-(1)-(e). Dr. Walters suggested the sentence be modified to read: "Has a protocol similar to the one proposed for a clinical trial been carried out in non-human primates and/or other animals? What were the results?"

Dr. Temin asked whether the word "malignancy" in Section I-B-2-c-(1)-(c) was the correct term. Dr. Mahoney pointed out that the working group might also wish to know if benign tumors develop. Dr. Motulsky suggested the working group substitute the words "development of neoplasia" for the word "malignancy" since neoplasia refers to both malignancies and benign tumors. The working group agreed to this proposed change.

Dr. Walters called the attention of the working group to Section I-B-2-c-(2) of the document. He said language asking how long the animals have been studied after treatment had been added to this section. The working group agreed it was reasonable to add this language to the document.

Dr. Grobstein thought the phrase "particularly germ line cells" should be added to the first sentence of Section I-B-2-c-(2). That sentence would then read as follows:

> "If a non-retroviral delivery system is used: What animal studies have been done to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ line cells)?

The working group agreed to this proposed modification.

Dr. Walters then called the attention of the working group to Section I-B-3, Clinical procedures, including patient monitoring, of the document. He noted that language requesting information on other similar studies had been included in this section of the document. The working group agreed to this proposed modification.

Dr. Walters said three items had been transferred from Section I-B-3 of the previous version of the document (Attachment II) to create a new section entitled, Reporting Requirements. The working group agreed to this modification.

Dr. Walters said language dealing with autopsy had been included in Section I-B-3-f and IV-B of the document. Dr. Temin thought Section I-B-3-f should specifically require an autopsy. The initial human gene therapy protocols will be scientific experiments, and an autopsy should be performed in order to gain all possible information. Should the patient die, one would wish to know why the patient died. One would also wish to know how the therapy affected the patient. Dr. Temin said investigators cannot lose track of human gene therapy subjects.

Dr. Motulsky asked what type of information would be sought in an autopsy. Dr. Temin said the investigator would wish to know if the introduced DNA was indeed where it should have been. The autopsy would also pinpoint the cause of death. Dr. Grobstein said an autopsy should be performed should the patient die in order to gain information which might help other patients.

Dr. Walters said an autopsy might be against some people's scruples.

Dr. Mahoney said all consent forms have an escape clause for withdrawing from the experiment. The patient, however, should not be part of this experimental procedure if the parents and patient are not willing to permit an autopsy.

Dr. Mahoney said these types of experimental procedures are a true partnership between the patient and the investigator; individuals not willing to participat fully in the procedure should not volunteer as subjects. In the initial phases of a research project, the investigator can require patients consent to procedures which might not be required in later stages of a project. After the initial trial protocol phase has been passed, individuals whose scruples prever them from participating in the earliest studies might be considered as subjects

Mr. Capron said the points to consider document should explicitly require the investigator be willing to speak to the parents or guardians about this aspect of the protocol. This issue must be on the table before the consent form is signed. The parents must have a moral understanding with the investigator.

Dr. Anderson said even though the consent form indicates a patient can withdraw from an experimental protocol, in some cases public health considerations prevent the patient from withdrawing from the protocol.

Dr. Motulsky asked whether state powers could be invoked to prevent a patient from withdrawing from the protocol. Mr. Capron felt they could be invoked since in some cases the patient cannot be permitted to withdraw.

Dr. McCarthy said he saw no problem in including an autopsy requirement in the points to consider document since it is not an absolute right to participate in research protocols. He said the working group could include language indicatin this section might be changed at a later date.

Dr. Lee asked whether the working group will set a time limit for an autopsy. Would the working group require an autopsy on an individual who has lived 20 or 30 years after treatment? Dr. Walters thought the points to consider docume would probably be modified within such a long time frame.

Ms. Areen and Dr. Temin agreed to develop language for this section of the document. They would focus on what special studies will be performed as part of the autopsy.

Dr. Walters then called the attention of the working group to the alternative language composed by Dr. Grobstein (Attachment V) for the Section, Public healt considerations. Dr. Grobstein said he particularly wished to ask whether any public health considerations associated with human gene therapy differ from

considerations with other therapies. Dr. Motulsky asked whether current Section I-B-4-c (Attachment IV) had addressed this consideration. Dr. Grobstein said he was specifically attempting to obtain probabilities on whether human gene therapy raises new public health concerns.

The working group agreed to incorporate the language proposed by Dr. Grobstein for Sections I-B-4-a, I-B-4-b, and I-B-4-c of the points to consider document.

Dr. Childress said an important public health consideration is whether hazards different from those that exist with other therapies are associated with human gene therapy; he suggested transmission of the viral vector might be one novel concern. He asked whether this concern should be spelled out in Section I-B-4-a. Dr. Rich thought the pertinent question was whether methods exist to mitigate risk to public health.

Mr. Capron proposed a Section I-B-4-d which would read: "What measures will be undertaken to mitigate the risks, if any, to public health?"

The working group agreed to this proposed modification of Section I-B-4 of the points to consider document.

Dr. Temin noted that one commentor had mentioned possible risk to phlebotomists and had suggested subjects of human gene therapy not be accepted as blood donors. He asked why these comments had not been incorporated in the points to consider document.

Mr. Capron said the issue is whether a large enough risk exists to stigmatize human gene therapy patients by including this type of language in the points to consider document. The subworking group did not feel such a risk exists. The working group agreed.

Dr. Walters called the attention of the working group to Section I-B-5-b which had been modified to include a question on patient follow-up. Dr. Motulsky asked if a time period had been set for follow-up. Dr. Walters said the intent is to request information on long-term care facilities. Dr. Grobstein said the word "reside" indicates the intent of the working group. Dr. Gottesman said the section, Reporting Requirements, specifies a three to five year follow-up period; she thought that time frame would be adequate.

Dr. Mahoney said this portion of the document simply informs the investigator that the patient should be followed for a period of time.

Dr. Walters called the attention of the working group to Section I-C-5 which deals with the fair selection of patients.

Mr. Capron said the points to consider should ask how will subjects be selected if more patients wish to be included in the study than the study can accommodate. He suggested the language read as follows: "How will patients be selected if it is not possible to include all who desire to participate?"

Dr. Walters called the attention of the working group to Section I-D, Informed consent, of the points to consider document.

Ms. Witherby felt the points in this section of the document should be statement rather than questions. Dr. Anderson felt this section of the document should be in the form of questions; individuals involved in clinical studies perform these types of activities as a matter of course. Mr. Mitchell said the working group would like to ensure, however, that this information is given to the patient.

Mr. Capron suggested the points in this section be questions but suggested they be prefaced by the word "how." The working group agreed to modify Sections I-D-1 and I-D-2 by adding the word "how."

Mr. Capron felt Section I-D-5 should be a declarative sentence. He thought specific language should draw attention to the working group's concern about the periods when the patient could withdraw from the protocol. Dr. Temin said the points to consider document should permit the investigator to discourage subjects from withdrawing from the protocol. Dr. Anderson suggested these important concepts be stated at the beginning of the section dealing with informed consent.

Mr. Capron said moral determination clauses exist, but the investigator has no means of forcing people to abide by an informed consent agreement even if a contract exists. Nonetheless, the investigator should convey to the patient the difficulty or danger of withdrawing from the study. The investigator might discuss why it would be difficult or inadvisable to withdraw from the study, and convey to the patient and guardians the moral responsibility the patient assumes in becoming part of these research protocols.

Dr. McCarthy said the consequences of withdrawing from a research protocol should be explained with moderation to the patient and the guardians. These consequences could be to the individual, to the institution, or to society as a whole.

Dr. Motulsky suggested the investigator should have the option of presenting this type of information to the patient.

Mr. Capron said the points to consider document could ask the investigator what information would be given to the subject on the topic of withdrawing from the protocol.

Dr. Mahoney said most current informed consent forms used with research protocols explicitly state these considerations. He said generally in clinical care, patients withdraw from care at their own risk.

Ms. Witherby suggested the following language be included in the points to consider document to read: "How will patients and/or their parents or guardians be informed of their right to withdraw from the proposed study, or the possibilit that at certain times it will be medically inadvisable to withdraw."

Dr. Walters said four issues should be addressed in this section of the document. These are: (1) the reversibility of the procedure; (2) conveying to the patient that at certain points in the protocol it is not advisable to withdraw from the study; (3) provision for long-term follow-up; and (4) autopsy.

Dr. Anderson thought human gene therapy would become irreversible when the transformed bone marrow cells are introduced into the patient.

Dr. Walters suggested the title of Section II might be modified to "Special Issues." Ms. Witherby questioned the rationale for this title change.

Dr. Grobstein felt the proposed title modification would change the emphasis. Dr. Rich said the word "social" should be retained in the title of this section. Mr. Capron agreed; he felt social issues do exist in the application of human gene therapy. The working group agreed to retain the title of "Social Issues."

Mr. Capron suggested the sentence indicating why the working group wishes to evaluate these issues should be deleted from the introductory paragraph in Section II. The working group agreed.

Dr. Walters called the attention of the working group to Section III of the document. Ms. Witherby suggested the working group request an abstract in lay terms. Dr. Temin agreed with this suggestion. Dr. Gottesman suggested the following language be added to Section III to read: "A one page description of the proposed experiment in nontechnical language."

Dr. Walters called the attention of the working group to Section IV of the document. Mr. Capron suggested the last sentence of Section IV-B dealing with autopsy should be modified. He suggested the word "when" be substituted for the word "if," since no patient will be immortalized by the procedure. Dr. Rich suggested the sentence should read: "In the event of a patient's death, the autopsy report should be submitted to the IRB and ORDA."

The working group agreed to this proposed language.

Dr. Grobstein suggested the telephone numbers be deleted from Sections IV-A and IV-B since he did not see the logic of including these numbers in these sections. Section IV-A requests that serious adverse effects be reported immediately; Section IV-B requests reports on the general progress of the patient. Ms. Witherby thought the document should request written reports be submitted to the Office of Recombinant DNA Activities (ORDA) as well as to the local Institutional Review Board (IRB) and the Office of Protection from Research Risks (OPRR). The working group agreed to these proposals.

Dr. Walters then called the attention of the working group to the language (Attachment VII) the subworking group had composed over lunch. He said this alternative language would be substituted for the third paragraph in the introduction.

Dr. Anderson suggested the first sentence should indicate a distinction "should be made" between genetic modifications in somatic cells and in germ line cells.

Dr. Motulsky suggested this sentence should indicate defective cells are treated in somatic cell therapy in the hope of inserting a properly functioning gene to aid the patient. He thought the document should clearly state the purpose of the procedure is the same as the traditional purpose of all clinical interventions, namely to benefit the patient.

Mr. Capron suggested the technique to be used, i.e., inserting a normal gene into bone marrow cells and reintroducing these modified cells into the patient, should be contrasted to germ line therapy. In germ line therapy, an attempt is made to change a subject's genes with the specific aim of changing the offspring genes. The working group agreed to this suggestion.

Drs. Temin and Motulsky questioned the inclusion of the sentence stating the working group at present will not entertain proposals for intentional germ line treatments but would review and approve somatic cell protocols. They argued that including such a statement would limit the working group's flexibility. They also pointed out that this language suggests the working group will approve of somatic cell proposals as a matter of course.

Dr. Anderson suggested the sentence should read: "The RAC and its working group will not at present entertain proposals for germ line treatments, but will consider for approval protocols for somatic cell gene therapy."

The working group agreed to this proposed language.

Dr. Motulsky suggested the language dealing with protocols "that satisfy the points raised in the following document" should be deleted since it also suggests the working group will approve of protocols as a matter of course. The working group agreed.

Dr. Temin said the word "human" should modify the phrase "somatic cell gene therapy."

Dr. Rich suggested the portion of the subworking group document (Attachment VII) which refers to public reports and hearings should indicate the points to consider are consistent with these reports and discussions.

Dr. Anderson added that the points to consider document has been widely circulated for public discussion; he suggested the low number of comments indicates some level of support for the position adopted by the working group.

Mr. Capron suggested the language referring to public hearings should be modified to read: "Concurring with this judgment, the RAC and its working group are prepared to consider for approval somatic cell therapy protocols."

Dr. Childress suggested the word "assurance" be substituted for the word "reassurance." Dr. Temin suggested the word "investigations" be substituted for the word "intervention."

Ms. Areen suggested language for the sentence dealing with undesired consequences as follows:

> "The two primary undesired consequences are unintentional: (1) vertical transmission of genetic changes from an individual to his or her offspring, or (2) horizontal transmission of viral infection to other persons with whom the individual comes in contact."

Dr. Temin said he found the language referring to germ line therapies to be very strong and too closely coupled to immediate somatic cell applications. Dr. Motulsky agreed with Dr. Temin; he felt this language should not be so strong since germ line applications at this time are hypothetical. Somatic cell applications would probably have some bearing on germ line applications, but this bearing is not direct and guaranteed.

Dr. McCarthy suggested the working group could simply indicate it will exercise vigilance over the application of data generated by somatic cell gene therapy protocols and not offer any statement on germ line applications. The document might indicate the working group will work with other bodies to generate a public assessment of the appropriateness of germ line applications.

Dr. Gottesman suggested the points to consider document might simply state that scientific developments in this, and other related fields may affect the development of technologies for germ line modifications.

Dr. Walters suggested the working group delete any reference to eugenics programs. Dr. Anderson said use of germ line therapy in eugenics programs is the biggest social issue associated with human gene therapy. He thought the document should refer to this potential application. Dr. Rich said the statement prepared by Dr. Walters (Attachment VIII) begins to approach this issue.

Mr. Capron suggested the working group might attempt to discuss the issues involved in germ line therapy; if the working group cannot address these issues, Congress may act to provide a forum for discussion.

Dr. Rich reiterated his suggestion that the working group develop a nontechnical document addressing technical and social issues. He said he would be willing to write a first draft of a position paper on the social issues associated with human gene therapy.

Dr. Anderson offered to provide a technical briefing to the working group at the next meeting of the working group.

Mr. Mitchell said the RAC would next convene on May 3, 1985. He said the points to consider document prepared by the Working Group on Human Gene Therapy will be presented to the RAC at that meeting.

Dr. Walters suggested the next meeting of the Working Group on Human Gene Therapy would be held in late spring or summer of 1985. Dr. Walters adjourned the meeting of the Working Group on Human Gene Therapy at 4:05 p.m.

[Executive Secretary's Note: The version of the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" resulting from this meeting is Attachment IX.]

Respectfully submitted,

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10/1/85
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LeRoy Walters, Ph.D.
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ATTACHMENT II OF THE MINUTES OF THE APRIL 1, 1985, MEETING OF THE WORKING GROUP ON HUMAN GENE THERAPY IS PAGES 2940 TO 2945 OF THE JANUARY 22, 1985, FEDERAL REGISTER WHICH CAN BE FOUND IN VOLUME 9 OF RECOMBINANT DNA RESEARCH.

ATTACHMENT III OF THE MINUTES OF THE APRIL 1, 1985, MEETING OF THE WORKING GROUP ON HUMAN GENE THERAPY APPEARS IN VOLUME 10 OF RECOMBINANT DNA RESEARCH AS ATTACHMENT IV TO THE MINUTES OF THE MARCH 15, 1985, MEETING OF THE SUBWORKING GROUP ON HUMAN GENE THERAPY.

ATTACHMENT IV OF THE MINUTES OF THE APRIL 1, 1985, MEETING OF THE WORKING GROUP ON HUMAN GENE THERAPY APPEARS IN VOLUME 10 OF RECOMBINANT DNA RESEARCH AS ATTACHMENT VI OF THE MARCH 15, 1985, MEETING OF THE SUBWORKING GROUP ON HUMAN GENE THERAPY.

of the principle investigator. If and when gene therapy protocols become more widely applied and use manufactured biological material, then the FDA publicati "Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by RDNA Technology," or the appropriate document at that time, should be consulted.

The clinical application of recombinant DNA techniques to human gene therapy raises two general kinds of questions. Part I of this document deals with the short-term risks and benefits of the proposed research to the patient[2] and to other people, as well as with issues of equity in the selection of subjects, informed consent, and privacy and confidentiality. In Part II, investigators are requested to address broader ethical and social issues pertaining to the research and its longer-term implications. These broader questions go beyond the usual purview of IRBs and reflect general public concerns about biomedical research. Part III of the "Points to Consider" summarizes other documentation that will assist the RAC and its working group in their review of gene therapy proposals.

The Working Group on Human Gene Therapy assumes that somatic-cell gene therapies need not differ in clinical terms from other accepted practices in medicine, such as organ or bone-marrow transplantation. It bases this assumption on such important public-policy deliberations as the Congressional hearing on "Human Genetic Engineering" (1982), the report of a presidential commission entitled Splicing Life (1982), and a recent Office of Technology Assessment background paper entitled Human Gene Therapy (1984). In light of the

[2]The term "patient" and its variants are used in the text as a shorthand designation for "patient-subject."

stated assumption, the working group judges that it is appropriate to review somatic-cell gene therapy protocols that may be presented at this time and to approve these protocols if they satisfactorily address the questions raised in the following document. However, in recognition of the social concern that surrounds the general practice of human gene therapy, the working group will, in its review of individual somatic-cell proposals, also consider the following issues: (1) is the proposed somatic-cell gene therapy a sufficient departure from existing medical therapies to require new risk-assessment and/or unusual precautions? (2) how strong is the evidence that the proposed somatic-cell gene therapy will not inadvertently affect reproductive cells? (3) does the proposed somatic-cell gene therapy contribute toward: (a) intentional germ-line gene therapy; (b) the enhancement of, rather than the correction of defects in, human capabilities through genetic means; or (c) governmental or other programs with eugenic objectives?

Responses to the questions raised in these "Points to Consider" should be in the form of either written answers or references to specific sections of the protocol or other documentation which accompanies the proposal.

I. Description of Proposal

A. Objectives and rationale of the proposed research

State concisely the overall objectives and rationale of the proposed study. Please provide information on the following specific points:

1. Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?

2. Describe the natural history and range of expression of the disease selected for treatment. In your view, are the usual effects of the

f. If a treated patient dies, will an autopsy be requested? If so, please indicate what special studies, if any, will be performed.

4. Public health considerations

Describe the potential benefits and hazards of the proposed therapy to persons other than the patients being treated. Specifically,

a. What potential public health benefits or hazards are postulated?

b. If there significant likelihood that the inserted DNA will spread from the patient to other persons or to the environment?

c. Will any precautions be taken against such spread (e.g., to patients sharing a room, health-care workers, or family members)?

d. Is the proposed procedure sufficiently different from existing therapeutic procedures to require special risk-assessment or risk-mitigating practices to protect public health? Why or why not?

5. Qualifications of investigators, adequacy of laboratory and clinical facilities

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of gene therapy. In addition, please describe the laboratory and clinical facilities where the proposed study will be performed.

a. What professional personnel (medical and nonmedical) will be involved in the proposed study? What are their specific qualifications and experience with respect to the disease to be treated and with respect to the techniques employed in molecular biology? Please provide curricula vitae (see III-D).

ATTACHMENT VI OF THE MINUTES OF THE APRIL 1, 1985, MEETING OF THE WORKING GROUP ON HUMAN GENE THERAPY APPEARS IN VOLUME 10 OF RECOMBINANT DNA RESEARCH AS ATTACHMENT V TO THE MINUTES OF THE MARCH 15, 1985, MEETING OF THE SUBWORKING GROUP ON HUMAN GENE THERAPY.

Federal Register

Monday
April 15, 1985

Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research: Availability of Environmental Assessment for Public Comment; Request for Comments on Need for a Programmatic Environmental Impact Statement; Notice

EPARTMENT OF HEALTH AND UMAN SERVICES

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ecombinant DNA Research; vailability of Environmental ssessment for Public Comment; equest for Comments on Need for a ogrammatic Environmental Impact atement

GENCY: National Institutes of Health, HS, DHHS.

CTION: Notice.

MMARY: This notice announces the ailability for public comment of the nvironmental Assessment and nding of No Significant Impact," dated nuary 21, 1985, "concerning the plication to the National Institutes of alth (NIH), under the NIH Guidelines r Research Involving Recombinant NA Molecules, of Drs. Steven Lindow d Nickolas Panopoulos of the niversity of California, Berkeley, to ld test ice-nucleation-minus bacteria epared by recombinant DNA chniques for purposes of biological ntrol of frost damage to plants." In dition, this notice requests comments the need for a programmatic vironmental Impact Statement in nnection with NIH approvals of oposals to release into the vironment organisms containing combinant DNA molecules.

TE: Comments must be received by ay 15, 1985.

DRESS: The January 21, 1985, vironmental Assessment and Finding No Significant Impact is available on a request to the NIH Office of combinant DNA Activities (ORDA), ilding 31, Room 3B10, National stitutes of Health, Bethesda, Maryland 205. Phone: (301) 496–6051. Comments ould be submitted to the same address d will be available for inspection and oying on weekdays between the hours 8:30 a.m. and 5:00 p.m.

R FURTHER INFORMATION CONTACT: William J. Gartland, ORDA, Building Room 3B10, National Institutes of alth, Bethesda, Maryland, 20205, (301) 1–6051.

PPLEMENTARY INFORMATION:

lackground

he NIH Guidelines for Research olving Recombinant DNA Molecules FR 46266, November 23, 1984) require t recombinant DNA experiments nducted at or sponsored by an titution that receives any support for ombinant DNA research from the tional Institutes of Health" (section I-f Guidelines) which involve "deliberate release into the environment of any organism containing recombinant DNA, except certain plants as described in Appendix L" (section III-A of Guidelines) must have prior approval from NIH before the experiment is conducted.

The NIH approved the application of Drs. Lindow and Panopoulous to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques on June 1, 1983 (48 FR 24549). Prior to the conduct of the field test, a lawsuit was filed challenging the approval on the grounds that NIH violated the National Environmental Policy Act (NEPA), (42 U.S.C. 4321 *et seq.*), and the Council on Environmental Quality (CEQ) regulations implementing NEPA (40 CFR Parts 1500–1508), by failing to prepare an Environmental Assessment (EA) or an Environmental Impact Statement (EIS) in connection with the approval of the field test.

On May 16, 1984, the United States District Court for the District of Columbia, in Civil Action No. 83–2714 (*Foundation on Economic Trends et al.*, v. *Margaret M. Heckler et al.*), issued a preliminary injunction which enjoined NIH from "approving or continuing to approve experimentation involving the deliberate release of recombinant DNA under * * * the * * * NIH Guidelines * * * until such time as the Court enters final judgment." The University of California was enjoined from proceeding with the Lindow-Panopoulos experiment "until such time as the Court enters final judgment."

The order of the District Court was appealed by the NIH and the University of California and on February 27, 1985, the United States Court of Appeals for the District of Columbia vacated the District Court's injunction as it applied to future approvals of deliberate release experiments by NIH, but upheld the preliminary injunction as it applied to the University of California experiment, concluding that the injunction should remain in effect until completion of an appropriate Environmental Assessment.

II. The Environmental Assessment

The NIH had begun preparation of an Environmental Assessment of the University of California experiment after the entry of the District Court order and that assessment and a Finding of No Significant Impact (FONSI) were completed on January 21, 1985.

This notice announces the availability for public comment of this 64-page EA–FONSI.

For convenience, the Table of Contents of the EA–FONSI

	Page
1.0 Introduction and Summary	1
2.0 Background Previous Administrative Action	3
3.0 Purpose and Need	8
3.1 Purpose of the Proposed Action	8
3.2 Need for the Proposed Action	9
4.0 Description of Proposed Action and Alternatives	11
4.1 Proposed Action	11
4.2 Alternatives to the Proposed Action	16
4.2.1 Approval of Original Proposal (September 17, 1982) Without Restriction	16
4.2.2 Approval of Modified Proposal (March 8, 1983) Without Restriction	16
4.2.3 Approval of Proposal as Approved by NIH on June 1, 1983, With Changes Proposed by the Investigators	16
4.2.4 Withhold Approval	17
5.0 Environment Effects	17
5.1 The Physical Environmental	17
5.2 Proposed Test Organisms	22
5.2.1 Characteristics of *P. s. syringae* and *E. herbicola* strains	22
5.2.2 Construction of INA Deletion Mutants	28
5.2.3 Previous Field Tests with INA Minus Bacteria	29
5.3 Effects of the Proposed Field Test	30
5.3.1 Risk Assessment	30
5.3.2 The Test Plot	34
5.3.3 Crops and Non-Commerical Plant Species	36
5.3.4 Local Epiphytic Bacterial Populations	37
5.3.5 Insect Populations	39
5.3.6 Animal Populations	41
5.3.7 Atmosphere and Climate	41
5.3.8 Scientific and Agricultural Benefits	45
5.3.9 Worst Case Considerations	46
5.4 Effects of Alternatives	48
5.4.1 Approval of Original Proposed Without Restriction	48
5.4.2 Approval of Modified Proposal Without Restriction	49
5.4.3 Approval of Proposal as Approved by NIH on June 1, 1983, With Changes Proposed by the Investigators	49
5.4.4 Withhold Approval	50
5.5. Summary and Conclusions	50
6.0 Consultation	52
7.0 References	53
Appendix A—Background Concerning the NIH Guidelines for Research Involving Recombinant DNA Molecules	57
Appendix B—Description and Location of Tulelake Field Station	B1
Appendix C—Diagram of Test Plot and Description of Treatments	C1

Comments on the EA and FONSI will be considered by the Director, NIH, when he decides whether to reapprove the field test proposed by the University of California.

III. The Need for A Programmatic Environmental Impact Statement

In its February 27, 1985, decision the Appeals Court stated that, "If NIH gives adequate environmental consideration to each deliberate release experiment . . . then we cannot, at this point, say that such consideration will obstruct environmental review. We thus reverse the District Court's finding that plaintiffs are likely to succeed in showing, at this point, that the governing law requires NIH to complete a programmatic EIS on deliberate release experiments." However, the Appeals Court went on to state that, "NIH should at least consider whether a programmatic EIS is required to fulfill the agency's legal obligations

under NEPA and the CEQ regulations." The NIH is initiating such a consideration and hereby requests comments on whether a programmatic EIS is required to fulfill NIH's obligations under NEPA and the CEQ regulations in connection with NIH approvals of proposals to release into the environment organisms containing recombinant DNA. The following information is provided to assist the public in commenting on this issue.

A. When is a Programmatic EIS Required?

In its decision of February 27, 1985, (p. 30 of the *Slip Opinion*) the Court of Appeals quoted a definition of a programmatic EIS from one of its previous decisions:

> A programmatic EIS reflects the broad environmental consequences attendant upon a wide-ranging federal program. The thesis underlying programmatic EISs is that a systematic program is likely to generate disparate yet related impacts. * * * Whereas the programmatic EIS looks ahead and assimilates 'broad issues' relevant to [the program], the site-specific EIS addresses more particularized considerations * * *. *Nat'l Wildlife Federation* v. *Appalachian Regional Comm'n*, * * * 677 F.2d at 888.

The Court of Appeals also referred (pp. 30–31 of the *Slip Opinion*) to criteria for determining when a programmatic EIS should be prepared:

> Under CEQ regulations a programmatic EIS should be prepared if actions are 'connected,' 'cumulative,' or sufficiently 'similar' that a programmatic EIS is 'the best way' to identify the environmental effects. 40 CFR § 1508.25. As this court has noted, 'Two considerations are especially helpful in reviewing the responsible officials' decision not to prepare a programmatic EIS: (a) Could the programmatic EIS be sufficiently forward looking to contribute to the decisionmakers' basic planning of the overall program? and (b) Does the decisionmaker purport to 'segment' the overall program, thereby unreasonably constricting the scope of * * * environmental evaluation?' *Nat'l Wildlife Federation* v. *Appalachian Regional Comm'n*, * * * 677 F.2d at 889. Thus a programmatic EIS should be prepared if it can be forward-looking and if its absence will obstruct environmental review.

B. Field Tests Approved by NIH

To date, NIH has approved three field tests of organisms containing recombinant DNA. One involved tomato and tobacco plants. When this was reviewed by the NIH Recombinant DNA Advisory Committee (RAC) on October 25, 1982, the statement was made that "the probability that the experiment would be successful is very low." In fact, the researcher found that the experiment did not succeed under laboratory conditions, and therefore for scientific reasons he does not wish to proceed with the field test.

This leaves only two cases of NIH approval for field tests of organisms containing recombinant DNA in which the investigators wish to proceed. One is the Lindow-Panopoulos experiment involving ice-nucleation-minus bacteria on which the January 21, 1985, EA–FONSI was prepared. The other, from Dr. Ronald Davis of Stanford University, involves corn plants (*Zea mays*) transformed with corn DNA. The environmental concerns raised by these two proposals are totally different and can only be meaningfully considered individually through an evaluation of the specific details of the proposed field test and the characteristics of the organisms proposed to be released, including their ecological niche, pathogenicity, viability and ability to escape from the field test site.

C. NIH's Role in the Approval or Disapproval of Proposals for the Release into the Environment of Organisms Containing Recombinant DNA

The Federal action for purposes of NEPA and the CEQ regulations is reviewing proposals for deliberate release experiments submitted to NIH by institutions which receive NIH funds for recombinant DNA research. There is no Federal program for the planning, conduct, and support of deliberate release experiments; there is only a Federal requirement that deliberate release experiments by NIH-funded institutions be approved by NIH.

This Federal requirement does not involve federally planned or initiated actions that are directed toward a particular objective. Proposals are submitted by different researchers from different fields at undetermined times for quite different experiments. Of the three proposals approved to date, two were not funded by NIH. They were submitted for approval because the institutions proposing to conduct the field tests received some NIH funds for recombinant DNA research.

The NIH's Guidelines are not "aimed at developing new technology which, when applied, will affect the environment." Rather, the Guidelines are aimed at scrutinizing the development of technology to ensure the safety of that development. In the course of this scrutiny of proposals for the field testing of organisms containing recombinant DNA, NIH has not identified any common environmental effect which could arise for the field tests that have been approved by NIH thus far.

The NIH does not know what proposals for deliberate release experiments may be submitted for approval in the future since most of the experiments are investigator initiated and are not planned by NIH.

D. NIH's Future Role in the Review and Approval of Proposals for the Deliberate Release Into the Environment of Organisms Containing Recombinant DNA

Until recently, the NIH was the only Federal agency reviewing and approving proposals involving the deliberate release into the environment of organisms containing recombinant DNA. That is no longer the case.

(1) On October 17, 1984, the Environmental Protection Agency (EPA) published in the Federal Register (49 FR 40659) and Interim Policy on Small Scale Field Testing of Microbial Pesticides which requires notification to EPA of proposed field tests involving certain microbial pesticides after which EPA will determine whether an Experimental Use Permit is required.

Advance Genetic Sciences, Inc. (AGS), had previously voluntarily submitted a proposal for NIH review and approval dealing with a field test of *Pseudomonas* strains containing recombinant DNA. The NIH gave notice of the upcoming review (49 FR 17672, April 24, 1984), and the proposal was considered at the June 1, 1984, RAC meeting. The NIH still had the proposal under review when AGS in a letter of February 11, 1985, withdrew the proposal from the NIH review process, deciding to rely only on EPA review under the October 17, 1984, Federal Register notice.

(2) On December 31, 1984, the Office of Science and Technology Policy published in the Federal Register (49 FR 50856) for public comment a Proposal for a Coordinated Framework for Regulation of Biotechnology. It includes a proposal to establish agency-based scientific advisory committees modelled after the NIH RAC in four other agencies (EPA, the Food and Drug Administration (FDA), the Department of Agriculture (USDA) and the National Science Foundation (NSF)).

If this new scientific advisory structure is established, proposals for deliberate release of organisms containing recombinant DNA that would have previously come to NIH for review and approval may go instead to EPA, USDA or NSF.

Dated: April 3, 1985.

ames B. Wyngaarden,

)irector, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official Government rograms contained in the Catalog of Federal)omestic Assistance. Normally, NIH lists in s announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program, but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected)

[FR Doc. 85–8798 Filed 4–12–85; 8:45 am]
BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

MAY 3, 1985

TABLE OF CONTENTS

Part		Page
I.	Call to Order and Opening Remarks	5
II.	Minutes of the October 29, 1984, Meeting	5
III.	Report of the Working Group on Release Into the Environment	5
IV.	Proposed Revision of Appendix C	9
V.	Proposed RAC Working Group	13
VI.	Report of Risk Assessment Subcommittee	18
VII.	Report of Working Group on Human Gene Therapy	19
VIII.	Proposal for a Coordinated Framework for Regulation of Biotechnology	26
IX.	Proposed Amendment of Part III of the Guidelines	31
X.	ORDA Proposal Regarding Receipt of Application Summaries	33
XI.	Future Meeting Dates	33
XII.	Announcement on a Symposium on Engineered Organisms in the Environment	33
XIII.	Adjournment	34

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING[1]

MAY 3, 1985

The Recombinant DNA Advisory Committee (RAC) was convened for its thirty-second meeting at 9:00 a.m. on May 3, 1985, in Building 31, Conference Room 6, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20205. Mr. Robert Mitchell (Chair), Attorney at Law in California, presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

Royston Clowes	Arthur Landy	Frances Sharples
Mitchell Cohen	David Martin	Anne Vidaver
L. Albert Daloz	Mark Mills	LeRoy Walters
Bernard Davis	Robert Mitchell	Pieter Wensink
David Friedman	Thomas Pirone	Anne Witherby
Susan Gottesman	David Pramer	William J. Gartland, Jr (Executive Secretary)
Irving Johnson	Fred Rapp	
Wolfgang Joklik	Mark Saginor	

A committee roster is attached (Attachment I).

Ad hoc consultant:

Gerard McGarrity, Institute for Medical Research

Non-voting members:

William Beisel, Department of Defense
George Duda, Department of Energy
John R. Fowle, Environmental Protection Agency
Philip D. Harriman, National Science Foundation
Morris A. Levin, Environmental Protection Agency
Henry I. Miller, Food and Drug Administration
Edwin Shykind, Department of Commerce
Sue A. Tolin, Department of Agriculture
William J. Walsh, Department of State

[1]The RAC is advisory to the NIH, and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

National Institutes of Health staff:

Stanley Barban, NIAID
Bobbie Bennett, OD
Robert Bonner, OD
Thomas Cloutier, OD
Becky Connors, NIAID
Irving Delappe, NIAID
Rosalind Gray, OD
Caroline Holloway, OD
Tateo Icho, NIADDK
Rachel Levinson, OD
Grace McDonald, OD
Robert McKinney, OD
Elizabeth Milewski, NIAID
Donald Ralbovsky, OD
Clifford C. Scharke, OD
Bernard Talbot, NIAID

Others:

Stanley H. Abramson, Environmental Protection Agency
Karen A. April, BioTechnica International, Inc.
Amy Bain, Lederle Laboratories
Frederick S. Betz, Environmental Protection Agency
Irene Brandt, Eli Lilly and Company
Steven Budiansky, Nature Magazine
Kathleen Canter, CBS Radio
Chia Ting Chen, Department of Labor
James F. Childress, Wilson Center, Smithsonian Institution
Isabelle Davidson, Pfizer, Inc.
Theodore J. DeLoggio, Pennwalt Corporation
Dominic D. Diascro, Jr., Pennwalt Corporation
Marie A. Dray, Pharmaceutical Manufacturers Association
Paula Dwyer, McGraw-Hill
Charles J. Eby, Monsanto Company
Neal Farber, Biogen Research Corporation
Peter Farnham, American Society for Biological Chemists
Gershon W. Fishbein, Environews, Inc.
Jeffrey Fox
Robert J. Frederick, Environmental Protection Agency
Luther Val Giddings, Office of Technology Assessment, U.S. Congress
Alan Goldhammer, Industrial Biotechnology Association
Carol Lax Gronbeck, Genentech, Inc.
Marlin Harmon, National Food Processors Association
Judith A. Hautala, Genex Corporation
Anne Hollander, Environmental Protection Agency
Don Irwin, LA Times
Susan James, NOVA
Dorothy Jessop, Department of Agriculture
Judy Johnson, Congressional Research Service

Roger S. Johnson
Geoffrey M. Karny, Finnegan, Henderson, Farabow, Garrett, and Dunner
John H. Keene, Abbott Laboratories
Martin Kenney, Ohio State University
Lorraine Kershner, Office of Assistant Secretary for Health, DHHS
Rihito Kimura, Kennedy Institute
Dennis Kopecko, Walter Reed Army Medical Center
Patrice Laget, French Embassy
Althaea Langston, Department of Agriculture
Robert Lamman, Office of General Counsel, DHHS
Warren Leary, Associated Press
Steven Lyons, Environmental Protection Agency
Kathryn R. Mahaffey, National Institute for Occupational Safety and Health
Jack J. Manis, Upjohn Company
Carl Mazza, Environmental Protection Agency
Julie Miller, Science News
Lorraine C. Minecci, Wyeth Laboratories
James Moe, Walter Reed Army Medical Center
David Moore, Association of American Medical Colleges
Claire Nader
Steve Olson, National Academy of Sciences
Vladimir Pakhomov, USSR Embassy
Sandra Panem, Environmental Protection Agency
Thomas L. Parker, Genetics Institute
Tabitha Powledge, Institute for Scientific Information
James Peterson, University of Virginia
Eleanor H. Peckham, Blum, Nash, and Railsback
Ronald A. Rader, Biotechnology Information Institute
Jeremy Rifkin, Foundation on Economic Trends
Patricia Roberts, Environmental Protection Agency
Marvin Rogul, The Rogul Group
Ronald L. Schaefer, ECOsystems International, Inc.
Harold Schmeck, New York Times
Mark C. Segal, Environmental Protection Agency
Smita K. Siddhanti, University of Pittsburgh
George P. Shibley, Department of Agriculture
Janet Shoemaker, American Society for Microbiology
William R. Srigley, Invitron
Paul E. Stern, University of Florida
Clarence E. Styron, Monsanto Company
Marjorie Sun, Science Magazine
Laura Tangley, Bioscience
Susan Thompson, Food and Drug Administration
Charles Turbyville, NIH Week
Joseph Van Houten, Schering-Plough
Vitolis E. Vengris, Food and Drug Administration
Lidia Watrud, Monsanto Company
Charles Weiner, Massachusetts Institute of Technology
Patricia Williams, Blue Sheet
Judith Wortman, American Institute of Biological Sciences
Stephanie Zobrist, Embassy of Switzerland

(Note: Page 4 is a blank page when the minutes are published in booklet form.)

I. CALL TO ORDER AND OPENING REMARKS

Mr. Mitchell, Chair, called the meeting of the Recombinant DNA Advisory Committee (RAC) to order. He said notice of this formal public meeting had been given in the March 28, 1985, Federal Register (50 FR 12456). He asked Dr. Gartland, Executive Secretary, whether a quorum was present. Dr. Gartland said a quorum was present.

Mr. Mitchell said in order to move expeditiously on a full agenda, he would recognize individuals in the following order: primary reviewers; other RAC members; ad hoc consultants to RAC; non-voting representatives to the RAC; RAC's administrative staff; members of the public who submitted written documents or comments; and finally other members of the public who wish to comment.

Mr. Mitchell welcomed several new committee members: Dr. Anne Vidaver of the University of Nebraska; Dr. Bernard Davis of Harvard University; Dr. David Pramer, of the Waksman Institute of Microbiology, Rutgers University; Dr. Mitchell Cohen of the Centers for Disease Control; and Dr. Irving Johnson of Eli Lilly and Company.

II. MINUTES OF THE OCTOBER 29, 1984, MEETING

Mr. Daloz said he and Dr. Saginor found the minutes (tab 1209) of the October 29, 1984, meeting of the RAC to be correct. He said that questions posed by the Assistant Secretary for Planning and Evaluation, Department of Health and Human Services (DHHS), had been well answered by the RAC Risk Assessment Subcommittee.

Dr. Saginor said the minutes were as lively to read as he remembered the meeting.

Dr. Gottesman moved approval of the minutes. The motion was seconded.

By a vote of twenty in favor, none opposed, and no abstentions, the RAC accepted the minutes of the October 29, 1984, RAC meeting.

III. REPORT OF THE WORKING GROUP ON RELEASE INTO THE ENVIRONMENT

Dr. McGarrity of the Institute for Medical Research, the Chair of the RAC Working Group on Release into the Environment, said he had made a preliminary report at the October 29, 1984, RAC meeting on the attempt of the Working Group on Release into the Environment to develop a points to consider document for field testing modified microorganisms (tabs 1202, 1203, 1204, 1216, 1217, 1218, 1219, 1227).

Dr. McGarrity said the working group's objectives were: (1) to develop submission guidance for investigators wishing to test microorganisms modified through recombinant DNA in the field; and (2) to facilitate RAC review.

Dr. McGarrity said the points to consider document begins with a general introduction to the topic of environmental testing. The first section of the document requests investigators to provide a general summary, to describe the objective(s) and the significance of the proposal, and to justify the proposal. The second section of the document requests information on the non-modified parental organism as well as information on the construction and molecular biology of the modified organism. The third section of the document requests information on environmental and ecological considerations. The fourth section of the document requests information on the conditions of the proposed field trial. The fifth section asks the investigator to provide a risk analysis.

Dr. McGarrity emphasized that the points to consider are general guideposts. Some of the points may not need to be addressed in a particular application; on the other hand, more information than requested by the points to consider may be required for some applications. Review of applications would be performed on a case-by-case basis.

Dr. McGarrity said these points to consider are not submitted for inclusion in the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules. The points to consider are intended to serve as guidance for investigators; these points should be flexible and modified as data accumulate from the testing continuum which extends from laboratory bench, to growth chamber or greenhouse, and to small-scale field testing.

Dr. Talbot, Deputy Director of the National Institute of Allergy and Infectious Diseases, said neither a vote by the RAC nor acceptance by the NIH Director is required for points to consider documents; RAC might, therefore, simply note this document as the current thinking of the RAC. The document could then be sent to individuals requesting it.

Dr. McGarrity said he would prefer to have RAC's administrative approval of the document.

Mr. Mitchell asked how a flexible oversight system could be developed with the great diversity of organisms, test sites, and techniques which could be used in field testing. Dr. Gottesman replied review must be performed on a case-by-case basis because of this great diversity.

Dr. Gottesman said comment letters (tabs 1216, 1227) had been received on the points to consider document in response to the March 28, 1985, Federal Register announcement. She suggested these comments be considered by the working group at its next meeting. Dr. Pirone said the issues raised in these comment letters could be handled rather simply by the working group

since the group has already discussed many of these points in detail. Dr. Vidaver agreed most of the points raised in the comment letters were considered by the working group in its extensive discussions.

Dr. Sharples said the points to consider document represents important compromises between a great diversity of opinion. She suggested the working group and RAC not introduce major changes in the document until the points to consider have been used to evaluate an actual field trial.

Dr. Johnson said he supported the points to consider document but suggested that in the future consideration be given to whether certain "innocuous" modified organisms could be exempted from review like "self-cloning experiments" in the laboratory.

Dr. Gottesman said the working group had discussed this possibility but could only agree that "information on all these points will not be necessary in all cases." She felt, however, the working group could perhaps attempt to distinguish cases which would not necessarily be exempt but which would not require a great deal of information for review. Dr. Gottesman said this approach would be in keeping with the manner in which the NIH Guidelines have evolved. Originally all experiments were covered by the NIH Guidelines. As information became available, requirements for some categories of experiments were reduced, and some categories of experiments were eventually exempted from review.

Dr. McGarrity reemphasized that modifications would be introduced into the points to consider document as data and experience accumulate. He expressed concern that this type of flexibility might not be present in oversight mechanisms proposed by other government agencies.

Dr. Pirone said although certain microorganisms in their natural state are innocuous, they may not be innocuous when altered. The interactions of microorganisms in nature are not well documented, and caution is appropriate.

Dr. Landy said he in principle supported attempts to develop categories of exempt organisms. However, no control would be applied over how exempt organisms would be introduced into the environment. Although a modified organism may be a variant of one occurring in nature, an investigator might disperse it in ways not normal in nature. While there may be no long-term environmental impact from such procedures, the possibility of transient effects should also be considered at this time. The working group approach is appropriate, and the document is currently an excellent guide for evaluating testing of modified microorganisms in the environment.

Dr. Clowes said the points to consider document represents a compromise between views of microbiologists and ecologists. Although some working group members felt some of the information requests were onerous and unnecessary, the correct approach clearly is to construct a flexible document to which individuals can respond as appropriate.

Dr. Gottesman said the working group expects the investigator to determine which points are important in the particular proposal and to provide relevant information to the working group and the RAC.

Dr. Davis said less information should be required for review if the modified organism is identical or virtually identical to something found in nature. He did not think RAC should develop a review system requiring a great deal of paperwork simply to relieve public anxiety over safe experiments.

Dr. Landy said RAC's attention to public concerns and anxiety has proven very beneficial; it is easier for RAC to modify its position than it is to reverse a court decision or legislation. Dr. Davis questioned whether widespread public concern actually exists or whether there is only a very vigorously expressed minority opinion.

Dr. Rapp supported Dr. Landy's comment; in order to build public confidence, RAC should proceed slowly and err on the side of caution so that the public feels environmental testing of modified organisms is being carefully monitored. As experience and information accumulate, RAC might consider creating exempt categories.

Dr. Gottesman said the distinction between the method of producing an organism and the product should not be lost: because an organism is produced using recombinant DNA techniques does not necesarily make it different from organisms derived by other means. A second issue is how environmental releases of any organism should be handled. Dr. Gottesman said: "...we run the danger of trying to solve every ecological problem in the world in the name of recombinant DNA and in most cases that is not really what the issue is."

Dr. Gottesman reminded the assembly that most genetic engineering using classical techniques and most transport of organisms from one location to another within the United States are not regulated.

Dr. Landy cautioned RAC to avoid situations which might unnecessarily impede recombinant DNA research. The public's perception of an issue is as important as the reality. If negative publicity is associated with transfer of an organism from one location to another, regardless of whether recombinant DNA is involved, that negative publicity may spill over to recombinant DNA research.

Dr. Gottesman thought RAC should begin to think about how to educate the public about these issues. She suggested RAC's discussion and the points to consider should serve as the basis for future considerations of environmental testing proposals. The points to consider document will evolve as experience accumulates.

Dr. Davis said about a dozen bacteria are now sold commercially for agricultural and other purposes. He agreed RAC should proceed cautiously but was concerned RAC might overreact to a perception of public concern and subject modified organisms, no more potentially harmful than those now on the

market, to an elaborate review process. He suggested organisms currently distributed commercially might be models for types of organisms to be considered for the exempt category.

Dr. Sharples said the Environmental Protection Agency (EPA) review process prior to approval of commercially available microbial pesticides is much more stringent than the process suggested in the RAC working group points to consider document. Mr. Fred Betz of the EPA corroborated Dr. Sharples' statement. He said: "EPA registration requirements are comprehensive in the area of product identity, human health considerations, environmental transport, and ecological effects." Historically, EPA has primarily reviewed large-scale experimental testing but recently has begun to evaluate microbial pesticides at the small-scale field testing stage.

Mr. Jeremy Rifkin of the Foundation on Economic Trends asked whether the recent decision (tab 1225) of the United States Court of Appeals for the District of Columbia Circuit had been addressed by the working group in developing the points to consider document. In developing the document did the working group address the issue of the adequacy of NIH environmental assessments.

Dr. McGarrity said the points to consider document is not designed to be an environmental assessment. In developing the points to consider, the working group considered many environmental concerns. The document represents the best scientific effort of this group of molecular biologists and environmental scientists.

Dr. Walters moved that RAC accept with thanks the points to consider document developed by the Working Group on Release into the Environment and request that in the future the working group submit to RAC any proposed major changes. Dr. Wensink seconded the motion.

By a vote of twenty in favor, none opposed, and no abstentions, the RAC accepted the motion.

Mr. Mitchell thanked Dr. McGarrity and the Working Group on Release into the Environment for an outstanding job. Dr. McGarrity thanked the EPA and the U.S. Department of Agriculture (USDA) for their significant contributions to this effort.

[Executive Secretary's Note: The document "Points to Consider for Environmental Testing of Microorganisms" is appended to these minutes as Attachment II.]

IV. PROPOSED REVISION OF APPENDIX C

Dr. Clowes introduced the proposal (tabs 1220, 1221) of Dr. Jack Manis of the Upjohn Company, Kalamazoo, Michigan. Dr. Manis had proposed that certain types of experiments involving Streptomyces fradiae and Streptomyces

lincolnensis be exempted under Section III-D-5 of the NIH Guidelines. The following language was proposed for inclusion in Appendix C of the NIH Guidelines:

"Experiments and processes utilizing recombinant DNA containing derivatives of Streptomyces fradiae or Streptomyces lincolnensis are exempt from the Guidelines at all levels of volume scale when the recombinant DNA molecules contained in these hosts are derived solely from nonpathogenic streptomycetes. The nonpathogenicities of the recombinant DNA sources are determined by the local Institutional Biosafety Committee (IBC).

"For these exempt laboratory experiments, BL1 physical containment conditions are recommended.

"For large-scale fermentation experiments BL1-LS physical containment conditions are recommended. However, following review by the IBC of appropriate data for a particular host-vector system some latitude in the application of BL1-LS requirements as outlined in Appendix K-II-A through K-II-F is permitted.

"Exceptions.

"Experiments described in Section III-A which require specific RAC review and NIH approval before initiation of the experiment.

"Experiments involving Class 3, 4, or 5 organisms (1) or cells known to be infected with these agents may be conducted under containment conditions specified in Section III-B-2 with prior IBC review and approval.

"Large-scale experiments (e.g., more than 10 liters of culture) require prior IBC review and approval (see Section III-B-5).

"Experiments involving the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates (see Appendix F)."

Dr. Clowes said a previous broader request by Dr. Manis had been rejected by the RAC at the February 6, 1984, meeting because sufficient background information had not been supplied. Dr. Manis has now limited his request and provided relevant information. The request cites the taxonomic relationships between these two Streptomyces strains and other Streptomyces strains and indicates there are only two pathogens in this genus; Streptomyces somaliensis which produces fungal-like infections in humans and animals, and Streptomyces scabies which produces a scab-like disease on potatoes and sugar beets.

Dr. Clowes found Dr. Manis' arguments persuasive. He said Streptomyces lincolnensis and Streptomyces fradiae have been used for more than twenty years in large-scale antibiotic preparation. Although Streptomyces lincolnensis and Streptomyces fradiae strains have not been cultivated for

as long a period or on as large a scale as Saccharomyces cerevisiae, they have, nevertheless, been cultivated at the greater than 100,000 liter scale for decades without any evidence of negative effects. Moreover, the experiments for which exemption is requested would involve recombinant DNA only from demonstrated non-pathogenic strains of other Streptomyces.

Dr. Clowes said he was reluctant at this time to move approval of Dr. Manis' request, however, because he would like to question Streptomyces experts on the interrelationships of the various Streptomyces species. There appears to be some contradiction between the data supplied by Dr. Manis and the positions of various Streptomyces species on Appendix A of the NIH Guidelines. He suggested Dr. Manis' request be referred to the Large-Scale Review Working Group for further study.

Dr. Pramer supported Dr. Clowes' suggestion to obtain additional information from experts on Streptomyces. Dr. Wensink agreed.

Dr. Friedman asked to be excused from the discussion as he has collaborative arrangements with investigators at Upjohn Company. Dr. Gottesman asked Dr. Friedman to contribute his expertise and knowledge to the discussion; he would not, however, vote.

Dr. Manis said Upjohn Company was requesting criteria for exemption, other than genetic exchange, for organisms traditionally used in large-scale experimentation in developing commercially usable novel antibiotics. He suggested nonpathogenicity might be a criterion. Dr. Manis said he hoped by this means to simplify review requirements.

Dr. Davis said the taxonomic criteria defining the Streptomyces are so "fuzzy" that relationships between species may be close by one set of criteria but distant by another set. He said he would consider nonpathogenicity a fundamental criterion. Safe cultivation of an organism on the 100,000 liter scale for a decade provides more than sufficient evidence of nonpathogenicity.

Dr. Henry Miller of the Food and Drug Administration (FDA) said FDA has a great deal of experience overseeing commercial uses of Streptomyces. He said FDA scientists have reviewed the proposal, and on theoretical and experiential grounds urge approval. He added that "the decision has the specific approval of Frank Young, the FDA Commissioner, who is a former RAC member and a microbiologist."

Dr. Johnson said the British Genetic Manipulation Advisory Group (GMAG) excluded all non-pathogenic strains of Streptomyces five years ago. GMAG in essence considered manipulations involving nonpathogenic Streptomyces self-cloning experiments. He thought the Upjohn proposal requested an action more limited in scope than the GMAG action, and he suggested the proposal be approved.

Dr. Gottesman said the current NIH Guidelines permit these experiments at the laboratory scale at Biosafety Level 1 and specify that investigators

must notify their IBC simultaneously with initiation of these experiments. At the large-scale level, IBC approval must be obtained before initiation of the procedure. Dr. Manis' request, therefore, deals primarily with the issue of whether investigators must consult their IBC before they initiate these types of large-scale processes. Dr. Gottesman expressed discomfort with a request to approve a blanket exemption for these organisms.

Dr. Gottesman said she had several questions on the Streptomyces. For example, what is known about the mechanism of pathogenicity? What might influence the behavior of nonpathogens? What is the frequency of genetic transfer? Is there any ecological concern about introducing novel antibiotic synthetic ability into an organism? She thought discussions with Streptomyces experts would be useful.

Dr. Manis said he had been unable to demonstrate genetic exchange with Streptomyces lincolnensis or Streptomyces fradiae or between those two strains.

Dr. Davis asked whether Streptomyces lincolnensis and Streptomyces fradiae could be placed on a sublist of Appendix A of the NIH Guidelines.

Dr. Gottesman said organisms are added to Appendix A when data demonstrate genetic exchange between the organisms. If the organisms exchange genetic material in the laboratory, such exchange is assumed to occur in nature. No genetic exchange has been shown with Streptomyces lincolnensis and Streptomyces fradiae, however, so they cannot be included on Appendix A.

Dr. Friedman said he saw no reason to change the current NIH Guidelines which require IBC approval before scale-up of such experiments.

Dr. Manis said Upjohn Company was requesting the flexibility in determining large-scale containment procedures accorded E. coli and S. cerevisiae host-vector systems be accorded to procedures involving Streptomyces lincolnensis and Streptomyces fradiae.

Dr. Robert McKinney of the NIH Division of Safety, the Chair of the RAC Large-Scale Review Working Group, said the NIH Guidelines provide sufficient flexibility to proceed at the large-scale level.

Dr. Gottesman said Dr. Manis' request should be referred to the Large-Scale Review Working Group for consideration.

Mr. Van Houghton, the Chair of Schering Corporation's Institutional Biosafety Committee (IBC), supported the opinion that these cases should be evaluated by the IBCs.

No motion was offered on the proposal, and Mr. Mitchell suggested RAC proceed to the next agenda item.

V. PROPOSED RAC WORKING GROUP

Dr. Martin introduced the proposal (tabs 1206, 1207, 1210) by Mr. Lee Rogers, Attorney for the Foundation on Economic Trends, and Mr. Jeremy Rifkin to create a RAC working group "whose stated purpose would be to examine potential uses of recombinant DNA technology for offensive and defensive biological weapons systems. In addition, this working group would explore current Department of Defense (DOD) programs specifically designed to develop 'defensive' preparedness against the threat of genetic engineering warfare by aggressor nations or terrorists....The working group may also wish to make recommendations regarding future oversight of recombinant DNA work in this field."

Dr. Martin said Mr. Rogers and Mr. Rifkin offer several justifications for creating this working group. One is that:

> "It is no longer possible to ignore the potential military uses of recombinant DNA experimentation in light of the DOD's plan to construct an aerosol test laboratory at Dugway Proving Ground in Utah."

A second is that:

> "The only area of recombinant DNA experimentation that has not yet been rigorously examined is the potential military uses.... this committee would find it helpful to explore the potential military uses of recombinant DNA technology in order to facilitate a better understanding of the various issues involved. Moreover, it is altogether appropriate for the RAC to engage in such a study as the DOD has stated on many occasions that it is adhering to the guidelines established by this committee and the NIH. An independent study by the RAC of the military potential of recombinant DNA technology can only serve to better inform the Executive Branch, Congress and the public of the issues involved in this particular field."

Dr. Martin said recombinant DNA technology can be used to obtain valuable information about biological processes; this knowledge could lead to the ability to alter these processes. The potential usefulness of recombinant DNA technology for biological warfare is, thus, comparable to its usefulness in human and animal health and in producing commodity chemicals and does not differ from other technologies in potential for warfare applications.

Dr. Martin said Secretary of Defense Weinberger's letter (tab 1206) to Congressman Sasser states the U.S. remains committed to the 1972 Convention on the Prohibition of Development, Production, and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction. He said a RAC working group would not be successful in obtaining non-public information from DOD. The executive and legislative branches have greater access to U.S. military recombinant DNA activities than does RAC. If these branches have questions concerning potential military use, RAC would be most willing to provide appropriate scientific expertise to evaluate these activities.

Dr. Martin said RAC has only formed working groups to examine areas where proposals have or will be submitted. There is no justification for forming a RAC working group to study potential military uses of recombinant DNA technology. RAC in the foreseeable future will not receive proposals involving the use of recombinant DNA technology in biological warfare.

Dr. Martin said:

> "What should RAC as a committee do? Nothing. What should we as individual scientists and citizens do? I consider that to be a matter that best be discussed before professional societies, a suggestion which the Foundation might note."

Dr. Walters suggested some RAC members might write as private citizens to Congress and request this issue be addressed.

Dr. Rapp said it is very difficult to verify that a biological system is being used for warfare; these difficulties are exemplified by the yellow rain issue in Southeast Asia.

Dr. Rapp said if one accepts DOD statements on its research programs, there is no need to explore this area; if one does not accept DOD statements, RAC is in no position to explore this area. RAC has neither the mandate, the resources, nor the budget to investigate these issues. He said he could not recommend RAC become involved in such an activity.

Dr. Gottesman said since the Asilomar Conference it has been recognized that this technology could be misused as well as applied to positive goals. This recognition is reflected in the structure of the NIH Guidelines which currently specifically require RAC review and NIH approval of certain types of proposals.

Dr. Gottesman said the question is whether RAC could do more to lessen the possibility this technology might be used for biological warfare. She thought RAC could do nothing more as a committee other than occasionally discuss this issue.

Dr. Davis said:

> "I'm as opposed to biological warfare as Mr. Rifkin and probably as suspicious of some aspects of military policy and I'm also aware of the fact that research for defensive purposes can be hardly distinguished from research that could conceivably someday contribute to offensive purposes...But I thoroughly agree...that this committee has no possible basis for trying to do anything useful in this field."

Mr. Rifkin said several recent developments should be considered by RAC. The Secretary of Defense went to Congress in August 1984 with an emergency request for funds to build a biological warfare aerosol test laboratory at Dugway Proving Grounds. Mr. Rifkin said the DOD Secretary indicated the

U.S. continues to obtain evidence the Soviet Union has maintained its offensive biological warfare program.

Mr. Rifkin said he had learned, but had not substantiated, that the Central Intelligence Agency (CIA) has been holding classified briefings for various agencies. At those briefings, the CIA is warning of a Soviet "threat" in genetic engineering. Mr. Rifkin said the chairman of a university molecular biology department had told him the CIA has been contacting molecular biologists to develop think-tank scenarios of possible uses of recombinant DNA technology in biological warfare.

Mr. Rifkin said the Department of the Army is using recombinant DNA technology in a cobra venom study. Several months ago several periodicals pointed out, but have not substantiated, that the Soviet Union was using recombinant DNA technology in research involving cobra venom. Mr. Rifkin said the DOD has received NIH permission to pursue this project; he asked RAC what is the redeeming social value of cobra venom research? He said: "Are there millions of people who potentially can be bitten by cobras around the world and therefore we need some kind of vaccine in order to inoculate them?"

Mr. Rifkin said RAC has examined every recombinant DNA technology application except biological warfare. He said RAC is "shirking" its responsibilities by not examining the ethics of using recombinant DNA technology in biological warfare.

Mr. Rifkin said he was suggesting RAC set up a working group to examine the technology and pose questions about the convertibility of this technology to biological warfare or military purposes. Such a mandate would not require investigation of DOD. Rather the working group would develop useful criteria to inform the public about the biological warfare implications of recombinant DNA technology. Mr. Rifkin said:

> "What has caused you in your collective minds to be so certain that there's absolutely no need for any other agency beside the Department of Defense to deal with the overall implications of recombinant DNA in this field. I just don't understand it and I'm really getting very frustrated and tired year after year of stone-walling and unanimous votes saying this committee has nothing to do with this technology because sooner or later you're going to have to deal with it. Sooner or later there's going to be a problem that's going to cause the Department of Defense to lock horns or the CIA or other agencies with this committee."

Mr. Mitchell said the United States has ratified the 1972 Biological Weapons Convention and RAC should not engage in any activity which might suggest the U.S. may be violating a treaty or is interested in violating a treaty.

Dr. Rapp said a computer can keep medical records but it can also be used to fire a missile. He said he could offer a good scientific rationale for studies involving cobra venom. Researchers should not be prevented from utilizing recombinant DNA technology simply on the outside chance some aspects could be converted to negative applications.

Dr. Davis said if DOD believes the Soviets are experimenting with cobra venom for biological warfare, it is not illegitimate for DOD to try to provide protection for the population. The NIH Guidelines address and permit the safe cloning of cobra venom toxin genes.

Dr. Gottesman said Mr. Rifkin requests that RAC develop scenarios for using recombinant DNA technology for biological warfare but is upset that the CIA is asking molecular biologists to develop such scenarios. She questioned whether any group could discuss scenarios without the possibility these scenarios would be used.

Mr. Rifkin said the difference between what he and the CIA was requesting was "like night and day." He said he was suggesting RAC:

> "...take a look at all of the potential implications for this technology so that you could draft appropriate guidelines and recommendations on how to best assure that we do not convert technology that was designed for one purpose to another unstated purpose."

Mr. Rifkin said he was not requesting RAC investigate violations of the Biological Weapons Convention. Rather he was requesting RAC study the potential military uses of this technology "so you can inform us on how we can safeguard ourselves so that this won't be used improperly."

Mr. Rifkin said RAC has evaluated the ethics of certain categories of recombinant DNA experiments. He said: "If the RAC committee has, and I know it has in the past, looked at the ethics of a particular type of experiment in relation to their risk, I don't understand why this kind of experiment should somehow be outside of its purview."

Mr. Rifkin said the NIH Guidelines deal with safety aspects of experiments. The DOD is in compliance with the safety aspects described in the NIH Guidelines but the ethics and merits of the experiments were not considered. The DOD may, thus, continue to perform all types of experiments as long as they are in compliance with the safety aspects of the NIH Guidelines. Mr. Rifkin said RAC will simply continue to say it has no responsibility. He said: "That seems to me to be not only unacceptable, but unconscionable from the point of view of the responsibilities of this committee."

Dr. Walters said RAC is not the appropriate committee for this type of evaluation. RAC is a reactive group. When a new application of recombinant DNA technology appears, RAC reacts by developing ground rules for that area of research. RAC has never performed studies of the type suggested by Mr. Rifkin.

Dr. Walters said Mr. Rifkin's proposal would create a working group of high visibility but very little substance. He suggested there were better ways for Mr. Rifkin to pursue his goals.

Dr. Johnson pointed out that RAC is not a regulatory authority or an investigative body. He thought RAC should continue to function as an advisory group.

Mr. Rifkin suggested RAC was "in a bind" because RAC has responsibility for approving DOD experiments but doesn't believe it has responsibility for overseeing or commenting on them. He suggested RAC indicate to DOD and Congress that RAC does not believe it should have the job of overseeing DOD experiments. He said:

> "...if this committee doesn't want to take a look at the implications of what it's clearing in terms of experiments, then it ought to just get out of the business of clearing experiments and give it back to the DOD or some other agency or the Congress to resolve."

Mr. Rifkin asked that a RAC member offer this suggestion as a motion.

Dr. Martin moved that RAC not establish a working group as requested by the Foundation on Economic Trends. The motion was seconded by Dr. Joklik.

Dr. Landy asked whether a motion was necessary. He asked Drs. Martin and Joklik whether they would consider the "no motion" option, i.e., offer no motion on the proposal.

Dr. Martin preferred to offer a motion because a motion indicates the request was seriously considered and a decision reached.

Dr. Davis moved to table Dr. Martin's motion. The motion to table was seconded.

By a vote of six in favor, thirteen opposed, and no abstentions, the RAC refused the motion to table Dr. Martin's motion.

Dr. Walters expressed concern that a motion that does not offer a rationale for RAC's conclusion may be misleading. He suggested a subgroup develop a rationale. Dr. Martin accepted this suggestion as an amendment to his motion.

Dr. Talbot asked whether the minutes of the May 3, 1985, RAC meeting could serve as the rationale. Mr. Mitchell expressed concern that the minutes will contain "a tremendous amount of material, some relevant and some perhaps irrelevant." The meeting was then recessed for lunch.

After lunch, Dr. Martin withdrew his motion; Dr. Joklik, the seconder of the motion, agreed.

There being no motion on the subject, Mr. Mitchell ruled the matter closed. He then asked Drs. Walters and Martin to assist him in considering whether a statement setting forth RAC's concerns about this issue should be developed.

Mr. Rifkin asked whether DOD would be required to submit classified experiments to the NIH for review. He asked if there are security clearance provisions for RAC members for review of classified materials.

Dr. Gartland replied that the vast majority of experiments conducted by DOD, as with any university or commercial concern, would be generically covered by the NIH Guidelines and would not be individually submitted to NIH for RAC review. He said the NIH Guidelines did not have any provisions for security clearance.

VI. REPORT OF RISK ASSESSMENT SUBCOMMITTEE

Dr. Gottesman said a series of questions (tab 1223) involving recombinant DNA risk assessment had originated in the Office of the Assistant Secretary for Planning and Evaluation (ASPE) of the Department of Health and Human Services (DHHS). This memorandum had been forwarded by the Assistant Secretary for Health, HHS, to several HHS agencies (Food and Drug Administration, Centers for Disease Control, and National Institutes of Health) for comment. The Director, NIH, suggested the Risk Assessment Subcommittee address these questions, and the Assistant Secretary for Health concurred with this proposal.

Dr. Gottesman, Chair of the Risk Assessment Subcommittee, said she had polled subcommittee members on the issues and had collated the responses in order to develop a preliminary draft response to the ASPE memorandum. The subcommittee met by telephone conference call (tab 1223) on October 15, 1984, to evaluate this preliminary draft response. Specific responses to the ASPE questions were also previously discussed at the October 29, 1984, RAC meeting (tab 1222).

Dr. Clowes said the Risk Assessment Subcommittee had addressed the question of whether the NIH Guidelines continue to be valid in light of new information generated in the past few years. The subcommittee agreed this information had been taken into consideration when the NIH Guidelines were designed or revised, and that certain sections of the NIH Guidelines were based on "worst case scenarios." The new information, thus, did not affect the validity of the NIH Guidelines.

Dr. Gottesman said the field of retrovirology is developing very rapidly and the subcommittee felt additional expertise should be sought to adequately address issues involving retroviruses. She proposed a discussion group with appropriate expertise be formed. The discussion group could attempt to "thrash out some of the issues" on retroviruses.

Drs. Friedman and Wensink agreed a discussion group on retroviruses would be helpful. Mr. Mitchell said any RAC members interested in participating in such a discussion group should contact ORDA.

VII. REPORT OF WORKING GROUP ON HUMAN GENE THERAPY

Dr. Walters, Chair of the RAC Working Group on Human Gene Therapy, said the working group had developed a guidance document (tabs 1201, 1213, 1214, 1215) entitled "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" (Attachment III).

Dr. Walters said this guidance document begins with an introduction to human somatic-cell gene therapy. Part I, entitled "Description of Proposal", requests first the objectives and rationale of the gene therapy protocol. The document subsequently poses a series of issues which parallel the general DHHS regulations for all research involving human subjects including research design, anticipated risks and benefits, selection of subjects, informed consent, and privacy and confidentiality. Part II of the document raises issues that are not usually evaluated by local review committees. One such issue is appropriate provision for sharing information with the public in a timely and accurate fashion. Part III of the points to consider outlines requested documentation and Part IV, reporting requirements.

Dr. Walters said the points to consider document had been published for public comment in the January 22, 1985, Federal Register; fifteen letters were received in response to this announcement.

Some modifications (tab 1213) have been introduced into the document in response to these comments. For example, the document's introduction more clearly accents the difference between somatic-cell and germ line gene therapy. The introduction reviews the multi-year process of public discussions concerning gene therapy. It now includes a quote from the December 1984 Office of Technology Assessment paper, Human Gene Therapy, which states:

> "Civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies."

Dr. Walters said the revised document now asks about information on prior laboratory studies in non-human primates and/or other laboratory animals. The phrase "and/or other laboratory animals" has been added to this version of the points, and indicates the working group's willingness to consider human gene therapy proposals on a case-by-case basis before the completion of laboratory studies in multiple species of animals.

Dr. Walters said the document has also been modified to request an agreement by prospective patients or their guardians to permit long-term follow-up and

an autopsy in the event of the patient's death. Both of these stipulations, in the opinion of the working group, are reasonable constraints on the autonomy of patients and their families in light of the benefits to future gene therapy patients.

Dr. Walters said some commentors had suggested the working group should possess additional types of expertise. The working group accepts this criticism but notes that no group of 15 people can "cover all the bases." The working group is committed, however, to obtaining additional expertise by consulting with ad hoc consultants as necessary.

Dr. Walters said the working group was presenting these points to consider not as a finished code of rules but rather as a checklist of the most important issues raised by the many thoughtful people who have discussed human gene therapy.

Dr. Walters said the working group will devote further study to two topics: (1) measures to disarm retroviral vectors; and (2) technical problems currently associated with germ line gene modifications in animal systems. In the future, the working group will devote detailed attention to specific ethical and legal questions surrounding gene therapy.

Dr. Walters then thanked the members of the working group, the liaison members, and ORDA staff for their efforts. He also thanked the individuals who commented on the points to consider document.

Dr. Walters identified a problem with the current version of the document: footnote one indicates the points to consider apply to both recombinant RNA and recombinant DNA. The current NIH Guidelines, however, do not officially cover recombinant RNA. He said Dr. Gottesman has a proposal for resolving this problem.

Dr. Gottesman proposed to modify footnote one of the points to consider document by eliminating the first sentence and the words "as well as to RNA" in the second sentence. The footnote would then read: "Section III-A-4 applies to both recombinant DNA and DNA derived from recombinant DNA." In addition, a proposal to modify the NIH Guidelines to cover RNA derived from recombinant DNA could be developed and published prior to the next RAC meeting.

Dr. Friedman said the working group did an excellent job in preparing the points to consider document.

Dr. Rapp said "the continuous request of certain individuals" to have a representative "of every stripe" on the Working Group for Human Gene Therapy is nonsensical. He said the major issues are how safe is the procedure and how can the interests of society be protected. The working group as currently constituted has been working well in dealing with scientific and social issues.

Dr. Davis specifically disagreed with the suggestion that a representative of industry be added to the working group; such an addition might give the mistaken impression that human gene therapy is or will soon be a large-scale industrial activity.

Mr. Robert Lamman, the NIH legal advisor, argued that the points to consider should not request a copy of the patient consent form. Dr. Walters said questions about the quality of consent forms have recently been raised for some innovative therapies. Mr. Mitchell said the working group was very sensitive about this issue and felt in the initial trials the consent form should be above reproach.

Dr. Rapp said human gene therapy protocols will be asking for agreements different from those requested in most clinical investigations. For example in human gene therapy protocols, the subject will be asked to participate in long-term followup and to give autopsy permission in advance. For this reason, he thought the working group should look at the consent forms for the initial gene therapy protocols.

Dr. Miller said FDA has several comments on the points to consider document. He said the introduction of the document does not accurately represent FDA's role in regulating these products. The introduction implies FDA jurisdiction will begin only when somatic-cell gene therapy protocols become more widely applied and use manufactured biological materials. Dr. Miller said FDA does not regulate gene therapy or technologies per se, but does regulate new drug products as defined in regulations. Dr. Miller said FDA has regulatory jurisdiction when a new drug product is used to effect human gene therapy, and FDA will indeed be overseeing human gene therapy experiments.

Dr. Miller proposed the following language be substituted for the last sentence of item (5) of the document's Introduction: "For information on the jurisdiction of the Food and Drug Admininstration over products employed for human gene therapy, please refer to the FDA's Statement of Policy."

Dr. Miller said item (8) of the Introduction misrepresents the aim of germ line therapy by stating the aim is "changing the set of genes passed on to the individual's offspring." He continued:

> "...in general, the object of gene therapy is to the cure the individual who will result from the embryo that is being manipulated. The effects on subsequent generations are usually a secondary effect and indeed the object is to cure that individual."

Referring to item (11) of the Introduction, Dr. Miller said "we believe [this item] states the question that any degree of teratogenesis is not acceptable." He said in conventional medical interventions. clearly that is not the case. Oblative radiotherapy before a bone marrow transplant, for example, for leukemia is considered acceptable. Oblative radiotherapy produces sterilization, and the risks to benefits in that situation are considered appropriate. Similarly, certain regimens of chemotherapy induce sterility or are clearly

teratogenic. Teratogenesis per se should not rule out a proposed medical intervention.

Dr. Miller said the document's request in Section I-B-3-e for information on the major anticipated potential beneficial and adverse effects of the treatment and on measures taken to control or reverse adverse effects "are really causes for speculation that's so complex as to be unreasonable."

Dr. Miller said the question in Section I-B-4-a of the document asking on what basis the potential public health benefits or hazards are postulated "is so ambiguous that it would be very difficult to answer and adds very little to the document."

Dr. Miller suggested a point H be added to Part III of the document. That point would ask:

> "What administrative official in the hospital, clinic or other institution has approved of this procedure?"

Dr. Miller said:

> "...we strongly believe...that since somatic cell gene therapy is an extension of present methods of therapy and is wholly analogous to conventional medical intervention, that decisions on individual protocols should be made in a way that is consistent with other conventional therapies; that is, primarily by physicians and scientists with expertise in the area. An NIH study section or an FDA advisory committee in a given discipline would not be composed largely of public policy specialists and bioethicists and attorneys. It would be comprised of clinicians and scientists with expertise in the area; and we feel strongly that the working group that evaluates such proposals should be comprised of persons largely with expertise in that area and not in these other areas as is presently the case."

Dr. Walters said the working group would like to obtain RAC's opinion on the April 15, 1985, version of the points to consider document. The working group will over the course of the next year be fine-tuning the document, and any major changes the working group might propose would be submitted to RAC for its consideration.

Dr. Walters felt the document should be circulated as soon as possible as representing RAC's best judgment on appropriate ground rules for this area of research and potential therapy.

Dr. Landy moved that RAC accept the points to consider document with appreciation. Minor changes can be made in the document without resubmitting the points to consider for RAC review. Significant changes would, however, be discussed by the RAC. This motion was seconded by Dr. Joklik.

Dr. Davis said he did not know if it is necessary to vote on this points to consider document.

Dr. Miller said FDA would be very disturbed if the document were issued, even in preliminary form, without first making some of the changes he requested since the current document "is grossly inaccurate." He said a perception already exists this version is the final version of the points to consider document; the magazine "Science" has indeed assumed incorrectly this version is the final document. Dr. Miller said:

> "I think that is an inaccurate representation and that's the kind of misinformation that is promulgated when corrections are not made and things are published prematurely."

Mr. Mitchell said the points to consider document was first published to obtain public comment in the January 22, 1985, Federal Register. No indication was given that this document was final.

Dr. Gottesman said she thought two changes could be made immediately: the changes in footnote one and Dr. Miller's first suggestion (for item (5) of the Introduction) concerning FDA's jurisdiction. She said the other changes suggested by Dr. Miller would require further discussion.

Dr. Walters said some of the modifications proposed by Dr. Miller had been discussed by the working group and a deliberate decision made not to accept them. Dr. Walters said he would not wish to incorporate Dr. Miller's proposed changes without the working group's knowledge or consent. He suggested the more substantive of Dr. Miller's modifications be sent to the working group for reconsideration.

Dr. Davis suggested the language concerning the purpose of germ line gene therapy be modified.

Dr. Gottesman said language discussing the purpose of germ line gene therapy can be ambiguous depending on whether one is thinking of the adult or the embryo. She agreed this language might be clarified but felt further discussion was necessary; she could not agree to a quick modification.

Mr. Mitchell agreed more discussion was required; he said RAC should avoid language which might cause confusion.

Dr. Davis suggested the points to consider document be returned to the working group for reconsideration.

Dr. Gottesman reiterated her suggestion that RAC approve the points to consider document as a working document with the two changes she had mentioned (i.e., in footnote one and in item (5) of the Introduction). Dr. Miller's other suggested modifications would be discussed at a future meeting of the working group. Dr. Gottesman said the points to consider document could be sent in the interim to investigators seeking guidance for proposal submission.

Dr. Landy said he could not agree with Dr. Davis' suggestion to return the points to consider document to the working group without RAC endorsement. He preferred a "working" document to a "perfect" document.

Mr. Mitchell said RAC could approve this points to consider document as a working document at this time with the understanding that modification is an ongoing process.

Dr. Miller said:

> "The only remaining problem we would have with that is that we feel that some of the nuances of wording and meaning that I described were not appreciated fully by the working group and by going back to them I think wouldn't be of much avail.
>
> "I think part of the problem of the working group takes me back to the last of FDA's points, that it's a group constituted largely of public policy people, lawyers and bioethicists with really a minority of clinicians and molecular biologists who don't understand completely some of these issues."

Dr. Landy asked whether Dr. Miller was requesting that RAC act as a board of appeal on the language of the document.

Dr. Miller said he was and said that: "we would prefer, of course, that this document is not promulgated in the interim with some of these errors still in them."

Dr. Saginor suggested the language on teratogenicity might be reconsidered since many drugs currently in use have teratogenic or other side effects. He suggested the document be approved as a working document, and the modifications suggested by Dr. Miller be evaluated carefully before the next RAC meeting.

Mr. Mitchell suggested the document could be approved as a working document which could be reviewed by the RAC at its next meeting. Dr. Saginor said this would be a reasonable approach.

Dr. Miller said he could not agree with this approach because of the "misconceptions" that result. He said: "I think again we haven't broached the issue of the composition of the group that will actually decide on these proposals which, as we all agree, are largely scientific and medical issues of prime importance."

Dr. Davis asked why RAC could not simply wait until the next RAC meeting to take action. He did not think investigators would be delayed if the document was not approved by RAC as a working document.

Dr. Gottesman disagreed with Dr. Davis' approach. She said the alleged "misconceptions" are not quite as dramatic as Dr. Miller paints them. Several of these comments were not made previously by Dr. Miller to the

working group in the form made at the May 3, 1985, RAC meeting, thus, it is not accurate to suggest the working group rejected these suggestions without careful consideration.

Dr. Gottesman thought not adopting the document would create problems. She said the question before RAC is whether this document is so drastically flawed it should not be adopted.

Dr. Walters said the differences of opinion existing between Dr. Miller and the working group cannot simply be attributed to differences between the scientific and nonscientific communities. Dr. Walters said distinguished scientists such as Drs. Howard Temin, W. French Anderson, and Arno Motulsky are members of the working group. These individuals have given a great deal of time and effort to developing the precise scientific language in the document. The document represents the best judgment of the working group on what is required to protect the first recipients of human gene therapy.

Mr. Mitchell said Dr. Miller had questioned the qualifications of the working group. He said RAC should satisfy itself that the working group possesses the requisite expertise. Mr. Mitchell said an effort had been made in constituting the working group to obtain expertise in a broad spectrum of disciplines. The intent was to obtain expert clinicians, scientists, ethicists, lawyers, and public policy people. The working group was constituted in this manner to address concerns raised by the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research.

Dr. Friedman said he did not see why Dr. Miller was so concerned with what "Science" publishes. He supported the suggestion that the points to consider document be used as a working document open to further revision.

Dr. Landy said he wished to modify his motion to emphasize that the points to consider would be a working document, and to acknowledge the FDA comments which will be considered at the next RAC meeting or by the working group.

Dr. Martin said the two modifications suggested by Dr. Gottesman could be introduced in the points to consider document and the document republished for comment. The RAC at the next meeting could respond to any comments received including Dr. Miller's comments.

Dr. Rapp supported this suggestion. He did not think RAC should override the working group but should ask them to reconsider these issues. Dr. Rapp suggested RAC accept the current points to consider as a working document. He said there is some urgency in developing a document which can be sent to investigators; he thought it would be a mistake to hold up the document for several more months when the current points to consider form a perfectly reasonable document. He called the question.

Dr. Landy restated his motion that the RAC accept with thanks the points to consider with the two modifications suggested by Dr. Gottesman (i.e., in

footnote one and in item (5) in the Introduction) as a current working document, republish it for comment, and consider it again at the next RAC meeting.

By a vote of twenty in favor, none opposed, and one abstention, the RAC accepted the motion. Mr. Daloz abstained.

VIII. PROPOSAL FOR A COORDINATED FRAMEWORK FOR REGULATION OF BIOTECHNOLOGY

Mr. Mitchell said this agenda item (tabs 1208, 1211) addresses a proposal for a coordinated framework for regulation of biotechnology. The December 31, 1984, Federal Register proposed a review mechanism for the oversight of biotechnology. The notice stated its purpose was to provide a concise index of U.S. laws related to biotechnology, to clarify the policies of the major regulatory agencies involved in reviewing research and products of biotechnology, to describe a scientific advisory mechanism for assessment of biotechnology issues, and to explain how the activities of the Federal agencies in biotechnology will be coordinated.

Mr. Mitchell said a special working group, the RAC Working Group on Biotechnology Coordination, had been formed to comment on the December 31, 1984, Federal Register notice. Mr. Mitchell, Chair of the Working Group on Biotechnology Coordination, constituted the group because the deadline for comment on the Federal Register proposal was April 1, 1985, while the next RAC meeting was May 3, 1985. He said the working group met for one day and attempted to offer some suggestions and observations.

Mr. Mitchell said several major points were made by the working group. For nearly ten years the NIH Guidelines have been the sole standard for the oversight of recombinant DNA experiments. Not only have scientists and industrial concerns in this country followed these NIH Guidelines, but other countries have adopted them as national standards. In addition, localities in the U.S. have based ordinances on the NIH Guidelines. The working group questioned how the proposal would affect countries and municipalities which have adopted the NIH Guidelines.

Mr. Mitchell said another concern was how the December 31, 1984, proposal would affect the flexibility of the NIH Guidelines. Until this time, RAC and NIH have had authority to modify the NIH Guidelines as knowledge accumulated. The working group feared such flexibility might be lost under the December 31, 1984, proposal.

Mr. Mitchell said the working group also expressed concern about public participation. The group asked whether the proposed advisory committees which would be established in other agencies would have public members and to what extent these members would participate meaningfully. In addition, in order for public confidence to be maintained, agency discussions should be open to public scrutiny. If the subject matter is fragmented between five different agencies, where would the public go to participate in discussions?

Mr. Mitchell said the working group felt the cooperation and confidence of scientific investigators must be maintained. A very bureaucratic and complex system would have a chilling effect on the scientific community. It is also necessary to maintain the confidence of industry and of the public.

Mr. Mitchell said the RAC working group suggested a distinction should be drawn between regulating products and regulating processes.

Mr. Mitchell said the working group also considered the structure of the proposed oversight mechanism. The issue appeared to working group members to boil down to whether a single agency approach or a federation of agencies is more efficient. A loose federation of agencies can work; the question is how the agencies will be coordinated and which agency will assure coordination. The more the working group considered the matter, the more it appeared a single review committee with appropriate working groups might be a better oversight mechanism. Mr. Mitchell said he had sent a letter expressing these working group concerns to the Cabinet Council Working Group on Biotechnology.

Dr. Gottesman said the most important points in the Working Group on Biotechnology Coordination discussion were: (1) Research should not be subjected to any additional regulation. The working group appeared to define research and application differently than the December 31, 1984, proposal. (2) How will the various agencies fulfill their oversight function? For example, will the agencies be required to review every document submitted? Will most reviews be performed by in-house agency staff? Would advisory groups only deal with policy? How the advisory groups are set up will differ depending on what is expected of them.

Dr. Clowes said the RAC working group viewed the proposed oversight mechanism as too complicated. The proposed mechanism adds an extra level of regulation and may lead to a lack of coordination and uniformity of review. The major problem, however, is that the proposed mechanism may not provide the continuity of experience RAC has provided. The Federal Register proposal would replace an effective organization with new and untried bodies which may need time to learn to function effectively. In the interim, a climate of insecurity may result; and this climate would likely have a negative effect on scientific progress and on its commercial application.

Dr. Clowes said he would like to offer the concept of an expanded RAC as a potential alternative solution to the complicated structure proposed in the December 31, 1984, Federal Register. Modifications could be made in the present structure and could take the form of additional working groups with greater cooperation and interaction from other agencies. These working groups might function as voting members of the RAC. This modified structure would retain the experience, confidence, and goodwill RAC has built up over the years. Dr. Clowes said he and several other members of the working group would prefer such a structure.

Dr. Friedman said the working group's primary concern is that research not be overregulated. Many members of the working group had a sense of deja vu in reading the Federal Register proposal and thought the wheel was being reinvented. Ten years of experience have permitted RAC to answer many of the questions raised in the December 31, 1984, Federal Register. Dr. Friedman said many members of the working group were concerned that RAC's collective experience be maintained and used.

Dr. Johnson said a clear contrast exists between the RAC system and the complexity of the proposed system. He felt the proposed system is redundant and will be inhibitory to the fragile U.S. lead in biotechology.

Dr. Johnson also thought the Federal Register proposal was written from the perception that products of biotechnology are "different" and that a different set of rules have to be designed to regulate and evaluate them. He did not think biotechnology products differed from products produced by other processes.

Dr. Johnson said the December 31, 1984, document contains policy statements by the FDA, the USDA, and the EPA. The FDA and the USDA essentially state that they possess sufficient authority to regulate products on a case-by-case basis in their area of jurisdiction. The EPA on the other hand appears to be attempting to regulate research. Moreover, they claim jurisdiction over products designed by processes that have been completely unregulated for many years, e.g., cell fusion, undirected, and site-directed mutagenesis. The EPA, thus, raises the spectre of handling research proposals differently if the ultimate intent is to commercialize. This is "regulation by intent." Dr. Johnson said the words "laboratory" and "experimental" should be defined and used consistently.

Dr. Pirone agreed and suggested the concept of laboratory be broadened to include all experimental protocols conducted in growth chambers, greenhouses, and perhaps even small-scale plots. Dr. Pirone said he was particularly concerned about how basic research in plant and animal sciences would fare under the December 31, 1984, Federal Register proposal. The proposal is vague about the review process; and research in plant and animal sciences might be overseen by USDA, EPA, NIH, or the National Science Foundation (NSF) depending on the considered intent of the experiments.

Dr. Walters said he had two primary concerns about the document published in the December 31, 1984, Federal Register. The first concern is a lack of emphasis on public discussion and debate at either the level of the scientific advisory committees or the oversight board. The Federal Register document does not contain a clear commitment to a public process. The second concern is that the document views too narrowly the mission of the advisory committees and the oversight board. In many cases, the advisory committees and the oversight board will be dealing with value judgments and not simply the gathering of scientific facts. Dr. Walters said these committees should possess not only the best scientific expertise available but also expertise in disciplines such as political science, law, and ethics.

Dr. Martin said the December 31, 1984, Federal Register proposal is an "enormous can of worms." He thought it posed significant threats to recombinant DNA technology, medical research, and the U.S. economy. He argued that if U.S. industries are not successful in first applying this technology and capturing markets, the U.S. will experience a loss of jobs, particularly for skilled and academically trained individuals.

Dr. Joklik agreed with Dr. Martin, and supported Dr. Clowes' suggestion; he thought expanded use of working groups and subcommittees under RAC's purview would be much preferable to the plan proposed in the December 31, 1984, Federal Register.

Dr. Pramer said a consistent feature of those comments he had seen is a recognition of RAC and its activities. The American Society for Microbiology (ASM) comment on the Federal Register proposal spoke in favor of continuing with a single review body rather than with a number of separate units. ASM expressed concern that differences in policy and procedures might exist with separate oversight bodies, and it wouldn't take long for individuals or institutions to find the path of least resistance.

Mr. Mitchell asked Dr. Talbot how RAC might appropriately continue to have a meaningful role in the development of oversight of biotechnology. RAC has a unique background of experience and should continue to play a role in this process.

Dr. Talbot said the comments made at the RAC meeting will be transmitted to the NIH Director. The Director represents the NIH at the Cabinet Council Working Group.

Dr. Talbot said if the other responses received by the Cabinet Council Working Group contain similar comments, strong pressure will exist to revise the proposed structure towards a single RAC or a modified RAC. Negotiations between the various agencies must be held.

Dr. Gottesman said it might be useful to have some formal discussion of the issues with representatives of the Cabinet Council. She said RAC would be eager to participate in an exchange of concerns.

Dr. Edwin Shykind who identified himself as a member of the Cabinet Council Working Group said he would report RAC's request. He said the RAC and the Cabinet Council Working Group should attempt to work together closely.

Dr. Carl Mazza of the EPA said the comments received in response to the December 31, 1984, Federal Register are being read very carefully by the various regulatory agencies.

Dr. Mazza said U.S. regulatory laws are complex, and it's no surprise that a complex document developed from the attempt to describe all of these regulations in one document.

Dr. Mazza said the EPA is not attempting to regulate research in so far as appropriate given the mandate of the law. In the December 31, 1984, Federal Register, EPA attempted to describe the implications and the limitations of the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide and Rodenticide Act.

Dr. Mazza said EPA has responsibility for regulating certain commercial products. The EPA mandate for reviewing these products should mesh with the historical role of the RAC. Some problems may develop with this transition. The EPA is very interested in making the fit between the EPA and the RAC work.

Dr. Mazza supported the suggestion that more opportunities for exchange of information be created.

Ms. Anne Hollander of the EPA, said EPA in the December 31, 1984, Federal Register discusses in great detail a number of different technologies and requests scientific comment on the risk implications of these technologies. She asked RAC to address the question of whether the different technologies have different implications for risk. Ms. Hollander contended RAC itself is based on the premise there is something different about recombinant DNA technology.

Dr. Davis said U.S. regulatory laws may be complex but RAC and the scientific community are not concerned with the law's complexity. Rather they are concerned with the complexity of the December 31, 1984, Federal Register proposal. He hoped it was crystal clear that a great many people do not think the proposed oversight mechanism is a good one.

Dr. Davis said there was a difference in tone between the section of the December 31, 1984, Federal Register written by the EPA and those sections written by USDA and FDA. He said FDA and USDA expressed considerable confidence in the way RAC has been handling the issues and were willing to rely on RAC for general advice for fulfulling their responsibilities. The EPA document on the other hand is much longer, and raises all types of questions. He said he had written a lengthy criticism on the EPA section of the Federal Register announcement. He did not address in that letter any of the EPA's hypothetical scenarios because the document's basic assumption is that these organisms are a terribly dangerous set of organisms. He said he felt as if he "was in about 1974." Dr. Davis said in 1985 there is not much reason from a scientist's point of view to assume these organisms are all terribly dangerous.

Dr. Davis said the assumption DNA is a toxic chemical to be regulated under TSCA puts the whole discussion in a framework that makes it extremely difficult for scientists to even want to start answering such questions.

Mr. Mitchell said the Working Group on Biotechnology Coordination did not have time to comment specifically on EPA's questions. Rather the working group focused primarily on the effect of the December 31, 1984, Federal

Register on the NIH, the NIH Guidelines, and the RAC. Mr. Mitchell suggested perhaps the working group might now review EPA's suggestions in greater detail and offer some response.

Dr. Davis did not think it appropriate at this time for the RAC working group to comment further on the December 31, 1984, Federal Register. He suggested RAC await the Cabinet Council Working Group response to the comments received.

IX. PROPOSED AMENDMENT OF PART III OF THE GUIDELINES

Dr. Friedman said this proposal (tab 1205) had been submitted by Dr. Talbot. Dr. Friedman said Dr. Talbot noted that under the NIH Guidelines certain proposals are received by NIH for review by RAC and subsequent NIH approval. Recently other Federal agencies have taken steps toward assuming new roles in review of recombinant DNA proposals. Because of these developments, proposals submitted to the NIH for RAC review may also be submitted to another agency for review.

Dr. Friedman said Dr. Talbot suggested it would be advantageous for NIH to have the option of deferring to other Federal agencies. In order to give NIH this latitude, Dr. Talbot suggested a new sentence would be added at the end of Section III-A of the NIH Guidelines just before Section III-A-1 as follows:

> "If experiments in this category are submitted for review to another Federal Agency, the submitter should notify ORDA; ORDA may then determine that such review serves the same purpose, and based on that determination, notify the submitter that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal agency."

Dr. Friedman said this proposal is an attempt to reduce paperwork. He supported Dr. Talbot's proposal.

Dr. Johnson felt the proposal did not clearly distinguish between oversight of products and oversight of experiments. He said he had no problem with Dr. Talbot's proposal if the proposal relates to regulation of products. He thought, however, that a single review body for evaluation of scientific issues should be in place.

Dr. Saginor asked whether Dr. Talbot's proposal referred to the review system proposed in the December 31, 1984, Federal Register or to the current system. Dr. Talbot said his proposal refers to the current system. For example, under the current system the Lindow/Panopoulos proposal involving the ice-minus bacteria was reviewed by both the NIH and the EPA.

Dr. Mills questioned whether other agencies would perform a type of review equivalent to RAC review. If review systems equivalent to RAC's exist, Dr. Mills said he would support Dr. Talbot's proposal.

Dr. Talbot pointed out that this proposal would give the NIH the option to defer to another agency. The proposal does not require the NIH to defer. The NIH currently does not have this option.

Mr. Clarence Styron of Monsanto Company asked whether Dr. Talbot's proposal had any implication for IBCs. Dr. Talbot replied it would have no implications for IBCs.

Dr. Miller said FDA feels: "there is now clear redundancy of oversight, between FDA and NIH, and USDA and NIH, and EPA and NIH, and FDA is disturbed by that."

Dr. Miller said while FDA supports the concept of relieving this redundancy, FDA did not think Dr. Talbot's proposal was the "optimal approach." FDA has three reservations: (1) there would be some uncertainty among individual investigators or industry about whether an exemption would be granted by ORDA; (2) some bureaucracy would be created at NIH and by the investigators or companies "to deal with correspondence, phone calls, meetings, requests to NIH;" (3) there is some ambiguity about the broadness of the exemption, i.e., if an investigator slightly changed his protocol, would he have to come back to NIH again.

Dr. Miller said:

> "...what we would prefer to see is some kind of categorical exemption so that an investigator who was being regulated by another federal agency would be exempt from oversight by NIH or perhaps it could be done more specifically so that oversight under specific federal statutes such as the Food, Drug, and Cosmetic Act, the Public Health Service Act, the USDA's Virus, Serum, and Toxin Act and perhaps others, EPA's statutes for example, would automatically be exempt from NIH oversight."

Dr. Miller said this type of exemption is done with TSCA; EPA is exempted from jurisdiction over products overseen by FDA's statutes.

Dr. Miller said he will formally propose this type of exemption in the Federal Register announcing the next RAC meeting and was now proposing that official action on Dr. Talbot's proposal be deferred until the next RAC meeting.

Dr. Sue Tolin of the USDA said USDA would like to see Dr. Miller's proposal of a categorical exemption be presented to RAC; and, therefore, feels approval of Dr. Talbot's proposal at the May 3, 1985, meeting would be premature particularly in light of the discussions occurring at this time in the Cabinet Council Working Group. She supported the request to defer action on this proposal.

Dr. Johnson moved that RAC table Dr. Talbot's proposal. Dr. Walters seconded the motion.

By a vote of seventeen in favor, none opposed, and no abstentions, the RAC accepted the motion to table the proposal.

X. ORDA PROPOSAL REGARDING RECEIPT OF APPLICATION SUMMARIES

Dr. Gartland said this proposal (tab 1212) developed from the December 31, 1984, Federal Register proposal for a coordinated framework for regulation of biotechnology. The Federal Register states:

> "Each agency will promptly send to its advisory committee a summary of each application relating to recombinant RNA, recombinant DNA, or cell fusion submitted to it for funding or administrative review, regardless of whether the agency is requesting a scientific review. The advisory committee may decline to receive summaries...."

Dr. Gartland said the number of summaries RAC would receive under this Federal Register language would be massive; thousands of such grant applications are submitted to NIH each year. He said RAC has never requested to see all of the grant applications submitted to the NIH. He proposed that the RAC decline to receive such grant application summaries since provision of such summaries to RAC would involve a tremendous workload with little or no benefit.

Dr. Walters moved that RAC accept the proposal. The motion was seconded by Dr. Gottesman.

By a vote of fifteen in favor, none opposed, and no abstentions, RAC accepted the motion.

XI. FUTURE MEETING DATES

Dr. Gartland said the next meeting of the RAC is scheduled for September 23, 1985. Mr. Mitchell said some working group meetings might also be held in the interim between the May 3 and September 23, 1985, RAC meetings.

XII. ANNOUNCEMENT ON A SYMPOSIUM ON ENGINEERED ORGANISMS IN THE ENVIRONMENT

Dr. Sharples said ASM is organizing a cross-disciplinary symposium entitled "Engineered Organisms in the Environment: Scientific Issues." This meeting will be held in Philadelphia, Pennsylvania, on June 10-13, 1985.

Dr. Sharples said the meeting is to identify the scientific issues and sort out the less important issues from the critical issues in this area. She urged interested individuals, including RAC members, to attend this meeting.

XIII. ADJOURMENT

Dr. Gottesman moved that the RAC adjourn. The motion was seconded. Mr. Mitchell ruled the May 3, 1985, RAC meeting adjourned at 4:15 p.m.

Respectfully submitted,

Elizabeth A. Milewski, Ph.D.
Rapporteur

William J. Gartland, Jr., Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

9/23/85
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Chair
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May 3, 1985

ATTACHMENT II OF THE MINUTES OF THE MAY 3, 1985, MEETING OF THE RAC IS ATTACHMENT V OF THE FEBRUARY 11, 1985, MEETING OF THE WORKING GROUP ON RELEASE INTO THE ENVIRONMENT WHICH CAN BE FOUND IN VOLUME 9 OF RECOMBINANT DNA RESEARCH.

ATTACHMENT III OF THE MINUTES OF THE MAY 3, 1985, MEETING OF THE RAC IS PAGES 2940 TO 2945 OF THE JANUARY 22, 1985, FEDERAL REGISTER WHICH CAN BE FOUND IN VOLUME 9 OF RECOMBINANT DNA RESEARCH.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

Memorandum

Date June 19, 1985

From Dr. Bernard Talbot, Deputy Director,
National Institute of Allergy and Infectious Diseases

Subject Analysis of Comments - ACTION

To Dr. James Wyngaarden, Director,
National Institutes of Health

I. Structure of This Memorandum

Section II of this memorandum contains background information. Section III analyzes public comments received and gives my recommendations in response to these comments. Section IV recapitulates the recommendations made in section III and provides a place for you to indicate whether you concur or do not concur with each recommendation. Section V discusses future action.

II. Background

On January 21, 1985, you signed your concurrence with an Environmental Assessment (EA) and Finding of No Significant Impact (FONSI) "concerning the application to the National Institutes of Health (NIH) under the NIH Guidelines for Research Involving Recombinant DNA Molecules by Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damage to plants." A copy of the January 21, 1985, EA-FONSI is attached at Tab A.

On April 15, 1985, there appeared in the Federal Register (50 FR 14794) a notice which you signed: (1) announcing the availability for public comment of the January 21, 1985, EA-FONSI; and (2) requesting "comments on the need for a programmatic Environmental Impact Statement in connection with NIH approvals of proposals to release into the environment organisms containing recombinant DNA molecules." A copy of the April 15, 1985, Federal Register notice is attached at Tab B.

In response to the April 15, 1985, Federal Register notice, fifteen letters were received. One of the letters, from Allan Hirsch, Director, Office of Federal Activities, Environmental Protection Agency (EPA), indicated that EPA had no comments to make on the EA-FONSI or on the need for NIH to prepare a programmatic Environmental Impact Statement (EIS). Copies of the other fourteen letters, which did comment on the EA-FONSI and/or the need for a programmatic EIS, are attached at Tab C.

III. Analysis of Comments

The fourteen letters at Tab C are analyzed below using the following structure.

III-A. Analysis of Comments on January 21, 1985, EA-FONSI
- III-A-1. Comments Praising the EA-FONSI
- III-A-2. Comments Criticizing the EA-FONSI
- III-A-3. Additional Comments and Response to Comments on EA-FONSI

III-B. Analysis of Comments on Need for Programmatic EIS
- III-B-1. Comments Indicating a Programmatic EIS is Not Required
- III-B-2. Comment Indicating the Need for a Programmatic EIS "Is Unlikely to Be Resolved in the Near Future"
- III-B-3. Comment Indicating a Programmatic EIS is Required
- III-B-4. Response to Comments on Requirement for Programmatic EIS

III-C. Comments and Response to Comments on Adequacy of April 15, 1985, Federal Register Notice

III-A. Analysis of Comments on January 21, 1985, EA-FONSI

Of the fourteen letters at Tab C, thirteen praised the EA-FONSI and one criticized the EA-FONSI.

III-A-1. Comments Praising the EA-FONSI

III-A-1-a. Public and Scientific Affairs Board, American Society for Microbiology

An April 24 letter from the Public and Scientific Affairs Board of the American Society for Microbiology (ASM) was signed by four individuals, i.e.,: Harlyn O. Halvorson, Ph.D., Chairman, Public and Scientific Affairs Board, ASM; David Pramer, Ph.D., Chairman, Committee on Environmental Microbiology, ASM; Monica Riley, Ph.D., Chairman, Committee on Genetic and Molecular Microbiology, ASM; and Kenneth I. Berns, M.D., Ph.D., Chairman, Committee on Medical Microbiology and Immunology, ASM.

The letter states:

> "The Environmental Assessment (EA) prepared by the NIH in relation to the experiments proposed by the Berkeley scientists is exhaustive in its coverage and persuasive in its arguments. It describes all proposed actions in detail and thoughtfully identifies and then considers the environmental effects of risks posed by those actions. We can think of no points to consider that are not addressed by the document. The agricultural history of the site is summarized. Climatological data is presented, and the animal and the human populations surrounding the experimental site have been surveyed. Reasonable consideration also was given to the probability of escape from the test site, to transport, and to

survival of the organism after escape. Finally, although not required, the EA concludes with worst case considerations that describe what is remotely or infintesimally possible, but highly unlikely.

"We concur in the opinion expressed in the EA that the proposed studies by the Berkeley scientists will have no significant impact on the environment for the following reasons: 1) No functional gene has been added to the bacterium. Rather, a single bit of chromosomal information has been biochemically deleted from the bacteria that will be applied to the test site; 2) Mutation of the same organism has been accomplished previously using techniques other than recombinant DNA, and deletion mutants have been demonstrated to occur naturally. Therefore, the organism produced by recombinant DNA deletion cannot be considered unique; and 3) Since the test organism does not contain a foreign gene and is not unique, it poses little or no added risk than that experienced under what are considered natural conditions.

"The EA makes a convincing case for the need for the proposed field test. We are certainly persuaded that the benefit of using ice-nucleation-minus bacteria will justify any risk that may be involved. Alternative procedures for protecting plants against frost damage require large amounts of energy and water or consume large quantities of fossil fuels, and they can create health hazards and adversely affect environmental quality. The Berkeley case offers certain benefits and limited risk, if any risk at all."

III-A-1-b. Dr. Donald Gustafson

A May 3 letter was received from Donald P. Gustafson, DVM, Ph.D., Professor of Virology, Department of Veterinary Microbiology Pathology and Public Health, Purdue University School of Veterinary Medicine. The letter states:

"This is to support and commend the statement made by the Public and Scientific Affairs Board of the American Society for Microbiology regarding the 64 page EA-FONSI related to the NIH approval of the application of Drs. Lindow and Panopoulous to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques (48FR24549). The statement is intelligent, concerned and powerful. It is from responsible, well informed scientists."

III-A-1-c. Dr. Winston Brill

An April 24 letter was received from Winston J. Brill, Ph.D., Vice President, Research and Development, Agracetus. The letter states:

"This document appears to be a thorough, balanced and accurate assessment of the environmental effects of conducting the proposed field test of ice-nucleation-minus bacteria produced through

recombinant DNA technology. Clearly a tremendous amount of thought and work has gone into its preparation. No documentation approaching this magnitude has previously been required for introduction to the environment of any microorganism, considered to be safe, that contains a man-made genetic alteration.

"Many individuals with diverse experience in areas relevant to the proposed project have evaluated potential environmental consequences of this experiment and have concluded that the chance of damage is sufficiently low that the experiment should no longer be delayed. The Environmental Assessment and Finding of No Significant Impact clearly indicates to the public that there is no cause for concern. The consultants identified at the end of the assessment include scientists who are well-versed with the issues and who have nothing personal to gain from the outcome of the experiments. These scientists are internationally recognized for their scientific judgments.

"Thousands of different mutant microorganisms have been released to the environment over a period of half a century. It is important to realize that there is no confirmed case of environmental damage caused by release of a safe microorganism that has been genetically modified in the laboratory. The Environmental Assessment strongly supports the conclusion that this experiment will not affect the environment in any substantively different way than the thousands of 'experiments' involving mutants of safe microorganisms that have already proceeded without problems."

III-A-1-d. Association of American Medical Colleges

A May 7 letter from the Association of American Medical Colleges (AAMC) was signed by John F. Sherman, Ph.D., Vice President, AAMC. The letter states:

"The Environmental Assessment prepared by the NIH in relation to the proposed Lindow/Panopoulos experiment is detailed and thorough. The National Institutes of Health and the NIH RAC are to be congratulated for establishing the procedures necessary to ensure proper review of proposed applications to conduct research which involve field testing or deliberate release into the environment of genetically engineered organisms. The establishment of the Working Group on Environmental Release of the Recombinant DNA Advisory Committee and the decision to prepare an Environmental Assessment statement on each such proposed experiment for deliberate release are both laudable parts of this process."

III-A-1-e. Zoecon Corporation

A May 8 letter from Zoecon Corporation was signed by Joseph P. Brown, Ph.D., Director, Toxicology & Regulatory Affairs, Zoecon Corporation. The letter states:

"Zoecon agrees with NIH's Comprehensive Environmental Assessment and finding of no significant impact with respect to the testing proposed by Drs. Lindow and Panopoulos. The facts that: (a) the INA- deletion mutant of the Pseudomonas syringae pv. syringae is isogenic with the naturally occurring P. syringae INA+ and contains no foreign DNA; and (b) that non isogenic INA- mutants produced through undirected mutagensis have been previously tested without adverse environmental effects; argue convincingly that the risk associated with the proposed field use is negligible. Also there is a finite probability that such isogenic deletion mutants have occured naturally in the past without notable effect and that the persistance and competitiveness of INA- mutants in general is insufficient to allow them to displace the wild type whose INA+ character probably confers a distinct competitive advantage for epiphytic colonization."

III-A-1-f. Genencor, Inc.

A May 10 letter from Genencor, Inc., was signed by Alice K. Jameson, Manager of Regulatory Affairs, Genencor, Inc. The letter discusses the January 21, 1985, EA-FONSI, but erroneously refers to it as an Environmental Impact Statement (EIS), which it is not, rather than an Environmental Assessment (EA), which it is. The letter states:

"We . . . find it to be thorough and well done and we agree with the finding by NIH of no significant impact."

III-A-1-g. Dr. C. Elizabeth Castro

A May 9 letter was received from C. Elizabeth Castro, Ph.D., Southwest Foundation for Biomedical Research. The letter states:

"It appears that the National Institutes of Health in initially reviewing the application of Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques and in issuing an Environmental Assessment and a Finding of No Significant Impact has fulfilled its mission."

III-A-1-h. Dr. Anne K. Vidaver

A May 13 letter was received from Anne K. Vidaver, Ph.D., Professor and Head, Department of Plant Pathology, University of Nebraska. The letter states:

"Relative to the Environmental Assessment for the proposal to field test ice-nucleation-minus bacteria, I find the document to be appropriate and comprehensive."

In addition the letter includes a number of specific comments on the EA which are cited and discussed below in Section III-A-3 of this memorandum.

III-A-1-i. Industrial Biotechnology Association

A May 19 letter from the Industrial Biotechnology Association (IBA) was signed by Harvey Price, Executive Director, IBA. The letter states:

> "The Industrial Biotechnology Association would like to express its agreement with the conclusion of NIH's enviornmental assessment in the ice-nucleation experiment case, that the modified field test proposal would have no significant environmental impact. We believe that the formal assessment makes clear that NIH's conclusion is supported by careful thought and abundant scientific evidence. While mindful of other public interests, the interest of encouraging such promising biotechnology research argues that NIH's substantial and conscientious effort to date should now be judged as fully adequate."

III-A-1-j. Lilly Research Laboratories

A May 14 letter from Lilly Research Laboratories (LRL) was signed by Irving S. Johnson, Ph.D., Vice President, LRL. The letter states:

> "There can never be an original experiment with zero risk. With admirable caution, the NIH-Recombinant DNA Advisory Committee (RAC) has recommended approval of experiments that carefully probe new areas of application of recombinant DNA technology. The field test proposal of Dr. Lindow and Panopoulos is such an experiment. The environmental assessment prepared by NIH summarizes current knowledge about all factors bearing on possible environmental effects; we concur with the logical conclusion that no significant environmental impact should result."

III-A-1-k. National Agricultural Chemicals Association

A May 15 letter from the National Agricultural Chemicals Association (NACA) was signed by Dr. Earl C. Spurrier, Vice President Regulatory Affairs, NACA. The letter states:

> "The NIH assessment appears to be well researched and documented. It represents a balanced, thorough and plausible evaluation of the environmental effects and impact of conducting research as proposed in the ice-nucleation-minus-bacteria project. It is apparent that all possible and relevant avenues of projected and potential concern were investigated and addressed. The document clearly goes beyond reasonable requirements to reassure the public that any possible field escape of the bacteria will have no adverse effects on or in the environment. The expert consultants who were involved are world-renowned for their scientific credibility and judgment. As such, the Environmental Assessment clearly supports the continuation of the research and the conclusion that this experiment will not adversely affect the environment in any substantive manner."

III-A-1-l. Dr. Mary Ellen Jones

A May 16 letter was received from Mary Ellen Jones, Ph.D., Kenan Professor and Chair, Department of Biochemistry & Nutrition, School of Medicine, The University of North Carolina at Chapel Hill. The letter states:

> "I write as the President of the Association of Medical School Departments of Biochemistry to state that as the principal elected representative of my society, I believe I can give their general support for the Lindow and Panopoulous field test of ice-nucleation-minus bacteria prepared by recombinant DNA techniques. . . .
>
> "I believe the most crucial issue is that natural deletion mutants occur so that the recombinant deletion mutants cannot be considered unique. . . . I believe the Environmental Assessment was exhaustive."

III-A-1-m. Dr. Trevor Suslow

A May 17 letter was received from Trevor Suslow, Ph.D., Director of Product Research, Staff Scientist Plant Pathology/Biocontrol, Advanced Genetic Sciences. The letter states:

> "In my opinion the EA is a well prepared document that involves the expertise of some excellent research plant pathologists and well qualified advisors."

Dr. Suslow includes one specific comment on the EA which is cited and discussed below in Section III-A-3 of this memorandum.

In addition, Dr. Suslow questions why the EA was prepared at all. He states that it has been "an unnecessary task asked of NIH," in which "much energy and effort was expended to restate the consensus" previously reached by the RAC at their April 11, 1983, meeting when they recommended approval of the Lindow-Panopoulos field test. The letter states:

> "I believe I also understand the basis for the requirement to complete such a document and Finding of No Significant Impact to satisfy legal requirements set for NIH. However, in reading the EA I was dismayed to find that such a large effort was expended to compile information that in my mind gives no greater assurance of safety than was available in the proposal of Lindow-Panopoulos that was approved by RAC. Except for site-specific information for Tule Lake, no substantially new information is brought forward to alleviate fears by documentation of experimental results. As no actual data is included or cited for many of the broad statements as to microbial competition, population dynamics, pathogenicity, etc., the statements as to risk or safety have diminished credibility.
>
> "My point is simply that this has been an unnecessary task asked of NIH and that the consideration and approval of the proposal by RAC, as submitted, was properly conducted with the currently available information in this subject area. The environmental and health

risks were properly evaluated with the finding of no foreseeable impact from this limited field experiment. Much energy and effort was expended to restate the consensus of the NIH-RAC decision."

III-A-2. Comments Criticizing the EA-FONSI

One letter was received criticizing the EA-FONSI. It was a May 14 letter from Mr. Edward Lee Rogers. Mr. Rogers is the lawyer for the plaintiffs in the suit, Foundation on Economic Trends et al. v. Heckler et al. (Civil Action No. 83-2714 in the U.S. District Court for the District of Columbia.) This case is mentioned both in the January 21, 1985, EA-FONSI and in the April 15, 1985, Federal Register notice. Mr. Rogers writes that the comments in his May 14 letter, "are submitted on behalf of the Foundation on Economic Trends, Inc., and the other plaintiffs in Foundation on Economic Trends v. Heckler." Attached to Mr. Rogers' May 14 letter are two declarations: one dated March 14, 2985, from Dr. David Pimentel, Professor of Insect Ecology and Agricultural Sciences, Cornell University; and one dated March 11, 1985, from Dr. Stephen Richard Gliessman, Associate Professor, Environmental Studies, University of California, Santa Cruz. The comments of Mr. Rogers, Dr. Pimentel and Dr. Gliessman are cited and discussed below in Section III-A-3 of this memorandum.

III-A-3. Additional Comments and Response to Comments on EA-FONSI

III-A-3-a. Dr. Anne K. Vidaver and Dr. Trevor Suslow

III-A-3-a-(1). In her May 13 letter, Dr. Vidaver makes some specific suggestions concerning, and proposes some specific alternative language for, the January 21, 1985, EA-FONSI, as follows:

"Page 22. 5.2.1.A.1. Nomenclature & taxonomy. Pseudomonas syringae is not an 'abbreviated taxonomic designation of Pseudomonas syringae pathovar (pv.) syringae' (see e.g. Bergey's Manual of Systematic Bacteriology, Vol. I. 1984, p. 168). Rather, P. syringae is a taxon with nomenclatural status according to the International Code of Nomenclature of Bacteria (1976); infrasubspecific taxa, such as pathovars, do not have such a status and are not part of the taxonomic hierarchy. The term pathovar is used to refer to a strain or set of strains with the same or similar characteristics, differentiated at the infrasubspecific level from other strains of the same species or subspecies on the basis of distinctive pathogenicity to one or more plant hosts (Dye et al. 1980; Rev. Pl. Pathology 59:153-168). That is, it makes no sense and is confusing to create pathovar names for sub-populations of species where there is no evidence that they have any pathogenic character. Thus, the epithet P. syringae is correct, since the strains being worked with are not and never have been shown to be pathogenic. An example of such correct usage is found in Gross, D. et al., 1983, Appl. Evn. Microbiol. 46:1370-1379. Those strains

of P. syringae that have been shown to be pathogenic may warrant the designation of pv. syringae or other pathovar epithet.

"Page 23. L.1. P. syringae and P. syringae pathovars are common plant epiphytes. None of these taxa has ever been shown....and do not grow...

"Paragraph two: L. 1. Certain strains or isolates of a pathovar of P. syringae (P. s. pv. syringae) have been...

"Page 24. Last paragraph: In summary, there are both non-pathogenic and pathogenic strains of P. syringae. Some strains of P. s. pv. syringae have...(This is analogous, for example, to the situation of the well-known Escherichia coli, which consists of both pathogenic and non-pathogenic strains. However, pathogenic strains of this bacterium are not given a latinized infrasubspecific epithet).

"Page 25. L. 3. P. syringae colonizes the leaf surfaces of different plants; some of these strains seem to be specific for certain host plants.

"Page 33. Paragraph one. It should not be implied that all naturally-occurring P. syringae have arisen due to deletion mutations. There is no evidence for this that I know of; i.e. it is likely that many, if not most, INA- P. syringae never were INA+ (e.g. see Gross, reference above)."

In his May 17 letter, Dr. Suslow makes a point very similar to Dr. Vidaver's first point, as follows:

"My only specific comment on the EA is that I disagree strongly with the use of Pseudomonas syringae pv syringae for epiphytic bacteria of this species which have no known pathogenicity to standard host plants. Until a host is found these strains could be referred to as P. syringae thus avoiding the confusion as to their pathogenic associations."

III-A-3-a-(2). My Response to Comments in Section III-A-3-a-(1)

I recommend that you accept the first proposed change given above in Section III-A-3-a-(1) of this memorandum, i.e., a proposed change on page 22 in the January 21, 1985, EA-FONSI, in the Section entitled "Nomenclature and Taxonomy." Although the January 21, 1985, EA-FONSI states that "the original and altered bacteria to be used in the experiment have been shown not to be pathogenic..." (page 2 of EA-FONSI), the change in text proposed by Dr. Vidaver makes even more clear the non-pathogenicity of the strains being worked with.

I recommend that you accept the second through fifth proposed changes given above in Section III-A-3-a-(1) of this memorandum, with the caveat that the fifth proposed change headed "Page 25, L. 3." is in fact a change proposed for the third line from the bottom on page 25 of the EA-FONSI, rather than the third line from the top. (I discussed this with Dr. Vidaver on the telephone and she agrees.) These proposed changes follow from the first proposed change given above in Section III-A-3-a-(1), and continue to clarify

that only certain strains of P. syringae (not being worked with in the proposed field test) are pathogenic.

I recommend that no change is necessary in response to the last proposed change given above in Section III-A-3-a-(1) of this memoranum. While I agree that "it should not be implied that all naturally-occurring P. syringae have arisen due to deletion mutations," I believe the January 21, 1985, EA-FONSI is clear on this point when it discusses "naturally occurring mutations, including deletions" (page 33, line 2 of EA-FONSI, underlining added.)

III-A-3-b. Mr. Edward Lee Rogers, Dr. David Pimentel and Dr. Stephen Richard Gliessman

III-A-3-b-(1). In his March 14 declaration, Dr. Pimentel states:

> "4. A statement is made that 'neither the original nor the altered bacteria are pathogenic to living creatures' yet in Lindow's published papers he refers to this bacterium Pseudomonas syringae (Ps) as an 'important pathogen' in nature. Lindow also speculates that Ps may well be pathogenic to insects. Thus, the statement in the assessment is incorrect.
>
> "5. It is stated that Ps 'did not induce frost or other injury to plants.' By plants is meant more than potato plants. Only a few tests were run with 6 crop species but no other crop plants that are cultured in that California region are included. Also no natural vegetation types were tested. Thus, insufficient data exist to support the quoted statement.
>
> "6. It is stated that 'certain strains or isolates of Ps have been reported to be pathogenic on many woody and herbaceous plants including bean, lima bean, wheat, barley, sorghum, corn, pear, apple, plum, peach, cherry, citrus, and others.' This statement contradicts an earlier quoted statement (see #4). Also, Lindow has not tested his Ps strain for its pathogenicity to all of these crop plants and again no natural plant species have been included in his environmental study.
>
> "7. It is stated that their strain of Ps 'will have been screened for pathogenicity towards oats, wheat, barley, corn, tomato, and three cultivars of beans.' When will these tests be completed and how will the scientific credibility of the data be validated? It should be noted that this list of crop plants makes up only a small portion of the total crop plants grown in the test area. In particular no fruit (pear, peach, plum, cherry, etc.) are included in the test nor are any natural plant species included.
>
> "8. It is stated that 'Analogous to plant frost survival, the presence of INA+ bacteria would be detrimental to survival of frost-sensitive insects and possible beneficial to frost-tolerant insect species.' This also contradicts an earlier statement that Ps is not 'pathogenic to living creatures' (see #4)."

III-A-3-b-(2). My Response to Comments in Section III-A-3-b-(1)

Dr. Pimentel writes that the following statement in the January 21, 1985, EA-FONSI is incorrect: "neither the original nor the altered bacteria are pathogenic to living creatures." The full quote (page 2 of EA-FONSI) is: "neither the original nor the altered bacteria are pathogenic to living creatures (insects, animals, birds, man)." This is a true statement. Pathogenicity of P. syringae to insects, animals, birds or man has never been shown. As Dr. Pimentel correctly points out, the EA-FONSI (page 23) states that certain strains of P. s. syringae (strains that will not be worked with in the proposed field test) "have been reported to be pathogenic on many woody and herbaceous plants including bean, lima bean, wheat, barley, sorgum, corn, pear, apple, plum, peach, cherry, citrus, and others." However Dr. Pimentel is incorrect in saying that the pathogencity of some P. s. syringae strains for some plants, contradicts the statement that the bacteria are not pathogenic to "living creatures (insects, animals, birds, man)." (Underlining added.)

Dr. Pimentel cites the statement that, "Analogous to plant frost survival, the presence of INA+ bacteria would be detrimental to survival of frost-sensitive insects and possibly beneficial to frost-tolerant insect species." (Page 40 of EA-FONSI.) This sentence in the EA-FONSI follows immediately a sentence that states that, "ice-nucleation-active bacteria may play at least a partial role in the limitation of supercooling of leaf-feeding insects." (Underlining added.) The key word here is "may." In response to Dr. Pimentel's point, I recommend that you change the sentence, "Analogous to ...." to read as follows: "If indeed ice-nucleation-active bacteria do play a role in the limitation of supercooling of leaf-feeding insects, then the presence of INA+ bacteria could be detrimental to survival of frost-sensitive insects and possibly beneficial to frost-tolerant insects."

In his point 7, given above in section III-A-3-b-(1), Dr. Pimentel quotes the phrase that the strains of P. syringae to be used in the field test "will have been screened for pathogenicity towards oats, wheat, barley, corn, tomato, and three cultivars of beans." (Page 36 of EA-FONSI.) He asks when the tests will be completed. In his points 5, 6 and 7, given above, he asks that tests also be done on other crop plants. In response to Dr. Pimentel's point, I telephoned Dr. Lindow and he indicated that additional tests have been completed. Therefore, I recommend that you change the sentence, "The strains to be used in the experiment will have been screened..." to read as follows: "The P. syringae strains to be used in the experiment have been screened, and have been found to be non-pathogenic, towards potato, oats, wheat, alfalfa, barley, cowpea, onion, horseradish, corn, tomato, three cultivars of beans (Buck blue lake, pinto and kidney), cherry, almond, pear, orange, and lemon."

As can be seen by comparing the list on page 18 of the EA-FONSI this includes all major crops grown in the area of the field test. In fact the EA-FONSI already contains on page 51 the statement that, "the strains to be used in the proposed field test have been isolated from healthy plants and screened to assure that they do not cause disease to major crops grown in the area."

III-A-3-b-(3). In his May 14 letter, Mr. Rogers states:

> "There was inadequate consideration of the nature of the modified Pseudomonas syringae ("Ps.) as highly competitive specimens chosen for their ability to displace their wildlife counterparts. Little or no attention was given to the genetic stability of these mutant bacteria, a point that Dr. Lindow emphasized distinguishes them from mutants created by chemical or radiation means. The implications for disruption of the natural balance between ice-nucleation-plus (INP) and ice-nucleation-minus (INM) Ps. is therefore not adequately considered."

III-A-3-b-(4). My Response to Comments in Section III-A-3-b-(3)

I do not agree with Mr. Rogers' statement that inadequate consideration was given in the January 21, 1985, EA-FONSI to these issues. The following are relevant quotations from the EA-FONSI:

> "The construction of INA- deletion mutants from INA+ isolated bacteria does not add new or exotic properties which would make the mutant more competitive than its parent bacteria. The mutation only deletes functions from the treated bacteria." (Page 28 of EA-FONSI.)

(Note that contrary to Mr. Rogers' characterization of the modified Pseudomonas syringae as "highly competitive specimens," the EA-FONSI specifically states that no new or exotic properties are added which would make the mutant more competitive than its parent bacteria. See also quote from Page 39 of EA-FONSI below.)

> "It is possible that small numbers of the INA- bacteria will be carried away from the test plot to surrounding crops or non-commercial plant species by wind, birds and insects. The numbers of bacteria involved would be very small in comparison to the wild-type bacteria that already exist in nature (P. syringae, for example is ubiquitous). It is highly unlikely that all of the deletion mutants that leave the test plot would be deposited in one place. Even if this did occur, there would be a negligible chance that there would be sufficient numbers of the deletion mutants to significantly affect the ratio between INA+ and INA- wild-type bacteria." (Page 36 of EA-FONSI.)

> "If the field test is successful, the bacteria that have had their ice-nucleation genes removed will colonize plants first and prevent the naturally-occuring INA+ from colonizing and becoming established on the plants, thereby protecting them from frost damage. The modified bacteria will be no more competitive, and probably less so, than their naturally-occuring INA+ counterparts because they are adapted only to the target crop for the experiment. It is believed that INA+ bacteria are more competitive than INA- bacteria because of their ability to induce frost damage and obtain nutrients through lesions caused by the frost damage. Because of this comparative lack of competitiveness and the small numbers of the modified bacteria in relation to the naturally occurring, ubiquitous bacteria, there is no significant risk that the modified bacteria will have an adverse

effect upon existing epiphytic populations." (Page 39 of EA-FONSI.)

"If, however, the modified bacteria were to escape the monitoring network and were to become established in neighboring fields, it is conceivable that the modified bacteria, together with INA- bacteria of the same or different species could alter whatever balance may exist in the particular field between INA+ and INA- bacteria. The scientific literature, however, does not indicate that there is any stable balance in the environment or in a field between INA+ and INA- bacteria. Large seasonal fluctuations and widely varying proportions of INA+ and INA- bacteria in a given field appear to be characteristic in nature, as bacterial populations change in response to environmental variables such as temperature and humidity. Among P. s. syringae, the INA+ phenotype may be dominant in one field at a given time, while the INA- phenotype is dominant in other fields in the vicinity, and the pattern may change over time with no perceptible environmental consequences, other than greater frost injury in INA+ fields. Existing agricultural practices add a further variable that drastically affects natural bacterial populations without any known adverse environmental consequences." (Page 47 of EA-FONSI.)

"Even if there were some fixed INA+/INA- balance in nature that had some environmental significance, it is unlikely that the slight alteration of that balance that could conceivably result if modified bacteria from this field test become established in adjacent fields would have any environmental consequences. The numbers to be applied are many orders of magnitude lower than the number of naturally-occurring bacteria, both INA+ and INA-, against which they would have to compete for space and nutrients for survival, growth and reproduction. Nothing in this proposed field test will enhance the reproductive potential or competitive fitness of the modified INA- bacteria. In all respects, it will be identical to its wild counterparts, except that it will be unable to ice nucleate. It will therefore be subject to the same environmental factors and the same population control mechanisms as the much more numerous wild bacteria. To the extent that the INA+ characteristic plays a role in growth, survival or reproduction, the modified bacteria will be more vulnerable to environmental and other stresses than its wild counterparts. Therefore, any escape of modified bacteria from the test site would not cause any significant environmental impacts." (Page 47-48 of EA-FONSI.)

"Large INA- populations of both P. s. syringae and E. herbicola already occur naturally. Depending on the host plant from which the bacteria are isolated, such bacteria can represent, for example, up to 80 percent for P. s. pv. syringae and up to 100 percent for E. herbicola of the total number of epiphytes present. Such large ratios of INA- bacteria in the environment suggests that some equilibrium already exists between INA+ and INA- strains and other bacteria. The populations proposed for field tests would be trivial introductions into the environment, and would not significantly affect that balance. Even if an imbalance in favor of the INA- bacteria did occur on a local field, a situation that already exists in nature, the result would be protection of the plants from frost damage or no effect."

(Page 50-51 of EA-FONSI.)

I recommend that no change is needed in the EA-FONSI in response to Mr. Rogers' comments given in Section III-A-3-b-(3).

III-A-3-b-(5). In his March 14 declaration, Dr. Pimentel states:

> "Any proposed field experiments must be designed to limit dispersal of the released Ps organism. At the same time the field experiments should detail how dispersal and movement of the released Ps organism in air, soil, water, and various organisms like bees, other insects, and birds, will be monitored."

In his March 11 declaration, Dr. Gliessman states:

> "The assessment does not seem to deal with the question of what possible tests need to be designed or could be designed to ensure greater reduction or elimination of risk of adverse effects from release of the altered organism. Since this case could form a landmark decision for future tests of altered organisms, it is even more important that this first case serve as a model for the future."

III-A-3-b-(6). My Response to Comments in Section III-A-3-b-(5)

The EA-FONSI does state on page 14-15 that:

> "Survival, lateral dissemination, and population dynamics of INA- mutants and INA+ populations in the field and the specificity if any, of INA- and INA+ bacterial interactions on leaf surfaces will be studied in parallel. To facilitate these studies, the strains will be marked with antibiotic resistance mutations not used in clinical medicine so that they can be selectively reisolated. Measurements of the applied bacteria will be made at 10m, 100m, and 1km in all directions from the site of application. Measurements of bacteria carrying the appropriate genetic marker will be made before treatment and weekly for the first month after treatment. Thereafter, treatment will be at monthly intervals. If appropriately marked strains are not detected by standard dilution plating procedures these procedures will be repeated following standard enrichment procedures."

The EA-FONSI does state on page 17 that:

> "The test plots occupy approximately one acre on the 100-acre section of the field station and will buffered by 20 feet of fallow land and a ten-foot road on all sides. In addition, there will be a band of untreated plants around the edge of the 0.4 acre plots to protect the treated plants."

In order to provide more information, I recommend that additional text be added to the EA-FONSI to follow immediately the text just quoted. The new text, taken verbatim from the September 21, 1984, letter from Dr. Lindow to

Dr. Gartland (Reference Y of the January 21, 1985, EA-FONSI) would be as follows:

> "In addition to the sixteen rows of plants used in the experiment an additional six rows of plants will be used as 'guard' plants to protect against severe air movements, dust, and other intrusions into the plot. Surrounding the entire plot will be twenty feet of soil which will be maintained free of any vegetation by cultivation, and surrounding the twenty foot barren area will be a ten foot wide dirt road for which little or no vegetation would grow."

A diagram showing these aspects of the test plot is already in the January 21, 1985, EA-FONSI as Appendix C.

III-A-3-b-(7). In his March 14 declaration, Dr. Pimentel states:

> "Lindow has not run any tests with beneficial insects like honey bees. Bees provide pollination to over $20 billion worth of U.S. crops annually. Other beneficial insects play an important role in biological control of pests.
>
> "Lindow should run adequate environmental studies with other crop and natural plant species and insects in the laboratory before his unique Ps bacterium is applied to test plots in the field. Then sound environmental tests must be designed to check for potential ecological impacts on fruit and other crops, important natural plants, and beneficial insects. As Lindow states the Ps organism has potential as an 'important pathogen'; thus ample evidence exists that careful environmental studies should be run before Lindow's Ps organism is released."

In his March 11 declaration, Dr. Gliessman states:

> "It seems that all discussion of potential environmental hazards associated with the release of the altered bacteria depends on the minimization of risk and low probability of unexpected problems. The fact that this infers that potential problems are not wholly eliminated is reason for concern and greater caution in the possible release of the ice-nucleating negative organisms that have been altered with recombinant DNA techniques.
>
> "In the section of the assessment dealing with worst case considerations, the assumption again is made that potential problems are at a low probability, due to the presence already of ice nucleation negative bacteria in the environment. Before release takes place, it seems to me that it is necessary to gain further understanding of how a balance is maintained in nature between negative and positive bacteria. How do they shift? What factors promote one over the other? How can such information be used to ensure zero probability of problems once the altered bacteria are released? Have sufficient tests been performed to demonstrate that altered ice nucleation negative bacteria are exactly the same as naturally-occuring negative strains? It seems to me that a battery of tests of this type should be performed before release takes place."

III-A-3-b-(8). My Response to Comments in Section III-A-3-b-(7)

Dr. Pimentel is citing other experiments that could be done in the laboratory before the field test is allowed to proceed. Dr. Gliessman writes of wanting to "ensure zero probability of problems."

One can always do more and more experiments in the laboratory in order to become more and more convinced that a proposed field test is safe. However, I believe, as did all the members of the NIH Recombinant DNA Advisory Committee, and the majority of respondents to our April 15, 1985, Federal Register notice, that sufficient information exists today, without additional experiments, to make a judgment that the proposed field test poses no significant risk. I recommend that no change is needed in response to the comments given in Section III-A-3-b-(7).

III-A-3-b-(9). In his May 14 letter, Mr. Rogers states:

> "Again there was not an adequately interdisciplinary review of the experiment. Insofar as we have been able to determine, the scientists listed as consultants have not made direct research contributions in the discipline of ecology of microorganisms. Dr. Kelman, the one scientist whose experise borders on this area, has made his contributions primarily in the area of physiology of parasitism, expecially as regards bacteria. Another one is a virologist, and the others are predominantly genetic engineers. The assessment would have been improved had qualified ecologists in the relevant specialities been included as consultants. Issues can only be recognized and dealt with adequately if there are qualified experts undertaking the evaluation."

In his March 11 delaration, Dr. Gliessman states:

> "Those consulted in the preparation of this assessment did not include an ecologist or evolutionary biologist. I feel that a broader range of expertise would be very important, expecially since many of the aspects involved deal with ecological and evolutionary processes in the environment."

III-A-3-b-(10). My Response to Comments in Section III-A-3-b-(9)

The NIH has gone far beyond all requirements in involving consultants in the review of the January 21, 1985, EA-FONSI. Neither the National Environmental Policy Act nor the Council on Environmental Quality (CEQ) Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act (40 CFR 1500-1508), nor Part 30, Environmental Protection, of the General Administration Manual of the Department of Health and Human Services (HHS), require the NIH to involve any persons outside of NIH in the preparation or review of an EA-FONSI. The NIH nevertheless consulted all the persons listed in Part 6.0 of the January 21, 1985, EA-FONSI. This is a distinguished list of consultants. Dr. Kelman, a member of the National Academy of Sciences, and chairman of its Section on Basic Biology and Agricultural Sciences, in his curriculum vitae

lists his "major research area" as "studies on the ecology of bacteria..." Dr. Upper and Dr. Coplin have published research articles in the journal, "Applied and Environmental Microbiology."

Then, going even further, although neither the National Environmental Policy Act, nor the CEQ Regulations implementing it, nor the HHS General Administration Manual require it, the NIH, in the April 15, 1985, Federal Register, announced that it would accept public comments on the January 21, 1985, EA-FONSI. Among the public comments received the following are relevant. Dr. Gustafson wrote that the EA-FONSI involved "responsible, well informed scientists." Dr. Brill wrote of involvement of "many individuals with diverse experience in areas relevant to the proposed project." Dr. Spurrier wrote that "the expert consultants who were involved are world-renowned for their scientific credibility and judgment." Dr. Suslow wrote that "the EA...involves the expertise of some excellent research plant pathologists and well qualified advisors."

Mr. Rogers attached to his May 14 letter, declarations from Dr. Pimentel and Dr. Gliessman. I assume that had Mr. Rogers received additional declarations criticizing the EA-FONSI from ecologists or from others, he would have attached them also.

I believe that the NIH has gone far beyond all requirements in soliciting comments on the EA-FONSI, and that no further action should be undertaken in response to the comments given in Section III-A-3-b-(9).

III-A-3-b-(11). In his May 14 letter, Mr. Rogers states:

> "The environmental assessment on the application for the University of California experiment is inadequate. It contains many of the same shortcomings that were part of the NIH's approach to the issue in court.
>
> "The EA fails to justify the conclusion that the experiment will have no significant environmental impacts because it fails to consider adequately the risks involved. Accordingly, we request that the NIH withdraw the EA and the FONSI, conduct the additional experiments requested in these comments and make a new evaluation before issuing another EA. In light of the unresolved environmental issues and potential impacts involved, it would be preferable for the NIH to prepare an EIS on the experiment at the outset rather than going through the interim step of preparing an EA. Had the NIH done so at the outset, the EIS could have been completed by this time and it will undoubtedly prove to be necessary."

III-3-A-b-(12). My Response to Comments in Section III-A-3-b-(11)

Mr. Rogers states that the EA is inadequate and that is "contains many of the same shortcomings that were part of the NIH's approach to the issue in court." It should be noted that neither the District Court nor the Appeals Court has to date considered the adequacy of January 21, 1985, EA-FONSI.

Mr. Rogers criticizes the EA-FONSI; however, in contrast to Mr. Rogers' views, this memorandum contains, in Sections III-A-1-a to III-A-1-m, numerous detailed, cogent statements praising the EA-FONSI. Thus the EA-FONSI is referred to as: "exhaustive in its coverage and persuasive in its arguments" (American Society for Microbiology); "intelligent, concerned and powerful" (Dr. Donald Gustafson); "thorough, balanced and accurate" (Dr. Winston Brill); "detailed and thorough" (Association of American Medical Colleges); "thorough and well done" (Genencor, Inc.); "appropriate and comprehensive" (Dr. Anne K. Vidaver); "supported by careful thought and abundant scientific evidence" (Industrial Biotechnology Association); "well researched and documented... balanced, thorough and plausible evaluation....all possible and relevant avenues of projected and potential concern were investigated and addressed" (National Agricultural Chemicals Association); "a well prepared document" (Dr. Trevor Suslow).

I recommend that you reaffirm the adequacy of the Environmental Assessment that you signed on January 21, 1985. Suggestions for changes in the January 21, 1985, EA were given above in Sections III-A-3-a-(2), III-A-3-b-(2) and III-A-3-b-(6) of this memorandum. These are minor changes, which all provide further clarification of safety of the field test, and in no way change the central conclusions of the EA.

I recommend that you reaffirm the Finding of No Significant Impact that you made on January 21, 1985.

I recommend you reject Mr. Rogers' suggestions given in Section III-A-3-b-(11) that the NIH should withdraw the EA-FONSI, and should prepare an EIS on the experiment.

I recommend, based on the EA and Finding of No Significant Impact, that you indicate your intention, once the Court lifts the injunction prohibiting NIH from reapproving the Lindow-Panopoulos field test, to reapprove the field test under the alternative discussed in Section 4.2.3 of the January 21, 1985, EA-FONSI. However to be even more conservative, I recommend that you limit your approval only to Pseudomonas syringae, and not to include Erwinia herbicola.

III-B. Analysis of Comments on the Need for a Programmatic EIS

Of the fourteen letters at Tab C, ten indicated that is was not necessary for NIH to prepare a programmatic Environmental Impact Statement (EIS) in connection with NIH approvals to release into the environment organisms containing recombinant DNA molecules. One said the need for such a programmatic EIS "is unlikely to be resolved in the near future." One indicated that such a programmatic EIS was necessary. Two did not deal with the issue.

III-B-1. Comments Indicating a Programmatic EIS is Not Required

III-B-1-a. Public and Scientific Affairs Board, American Society for Microbiology

The ASM letter states:

> "We question whether an EIS is desirable or necessary in the specific case of the experiment by Drs. Lindow and Panopoulos to test ice-nucleation-minus bacteria prepared by recombinant DNA techniques. We also question whether a programmatic EIS should be required in any case where an adequate EA is provided. By definition, a programmatic EIS is concerned with broad issues rather than with specific considerations which relate to a particular site. In the Berkeley case, and it seems to us in all but the most unusual circumstance, an EA will serve as well as an EIS to identify environmental effects. The EA is more concerned than the EIS with the present. The EIS is more forward-looking than the EA, and the availability of an EA without an EIS will not necessarily obstruct environmental review. The absence of a programmatic EIS does not hinder a continuing concern for environmental effects.
>
> "The thoroughness of preparation of the EA and the fact that the organism to be tested by the Berkeley scientists does not contain a foreign gene and is not unique, obviates the need in this case for a programmatic EIS. If future EAs are as thoroughly and well prepared as the present document, there should be little need in general for programmatic EISs. Nevertheless, any experiment which proposes to take an organism from the laboratory and apply it in the field must undergo careful review, and the need for the review must be determined on a case-by-case basis. Given an EA, the decision concerning need for a programmatic EIS also should be made on a case-by-case basis. Every effort should be made to see that a programmatic EIS is not required if all it accomplishes is to duplicate what is already presented in an EA.
>
> "The PSAB applauds the NIH and the NIH Recombinant DNA Advisory Committee (RAC) for the way they have managed a difficult assignment very well. In conclusion, we believe that NIH has effectively dealt with this issue and that there is not further need for a programmatic Environmental Impact Statement."

III-B-1-b. Dr. Winston Brill

The letter from Dr. Brill states:

> "Man has had centuries of experience with plant breeding, a century of experience with genetically altering microorganisms, and half a century of experience with gene exchanges among microorganisms. No special concerns have been raised from release of organisms produced through man-made and man-selected genetic alterations. We consider these types of projects to be very safe; consequently, we have no regulations to govern release of genetically altered organisms which are regarded as safe.
>
> "In comparison to traditional genetic technologies, recombinant

DNA technology actually is more precise and predictable in its outcome. Thus, the level of concern about problems from recombinant DNA techniques should be no more than the level of concern associated with release of organisms developed using conventional techniques

"The NIH should not prepare a Programmatic Environmental-Impact Statement unless there is a scientifically sound reason why application of recombinant DNA technology will inadvertantly create a serious environmental problem. The fact that a Programmatic Environmental Impact Statement has never been deemed necessary for traditional practices such as breeding and mutation, strongly suggests that a Programmatic Environmental Impact Statement would not be useful for recombinant DNA work. Arguments must be made by proponents of a Programmatic Environmental Impact Statement to demonstrate that a recombinant organism indeed has a greater chance to create environmental damage than a genetically altered organism derived through traditional practices."

III-B-1-c. Association of American Medical Colleges

The letter from Dr. Sherman states:

"The Association strongly supports the argument...that a programmatic EIS is neither necessary nor appropriate for future research proposals involving field trials of genetically engineered microorganisms. A single a priori programmatic EIS cannot be made in regard to such experiments. They are not part of a deliberate NIH research program but rather unsolicited and as yet unknown proposed future experiments. Each experiment will be a unique instance which must be evaluated for environmental impact on a case-by-case basis. The Environmental Assessment (EA) statement admirably serves this purpose by providing a full evaluation of the environmental impact of a given proposed experiment involving field testing of a genetically engineered organism."

III-B-1-d. Zoecon Corporation

The letter from Dr. Brown states:

"With respect to the need for a Programmatic Environmental Impact Statement, Zoecon believes that in view of the increasing responsibilities of other Federal agencies notably the Environmental Protection Agency, such programmatic EIS should not be required of N.I.H."

III-B-1-e. Genencor, Inc.

The letter from Ms. Jameson states:

"Further, from our understanding of the programmatic EIS and the varied nature of recombinant organisms and environments in which they may be released, we feel that the assessment of their environmental impact can best be determined on a case-by-case basis... rather than a programmatic EIS."

III-B-1-f. Dr. C. Elizabeth Castro

The letter from Dr. Castro states:

"The preparation of a programmatic Environment Impact Statement (EIS) seems unnecessary, particularly as its absence would not appear to obstruct environmental review."

III-B-1-g. Dr. Anne K. Vidaver

The letter from Dr. Vidaver states:

"I do not see a need for a programmatic Environmental Impact Statement since anticipated proposals and actions by RAC are not connected, cumulative or likely to be sufficiently similar, criteria which I understand are considered standard for such an EIS."

III-B-1-h. Industrial Biotechnology Association

The letter from Mr. Price states:

"We also concur... that a programmatic environmental impact statement would not be appropriate in connection with environmental release approvals at this time...."

III-B-1-i. Lilly Research Laboratories

The letter from Dr. Johnson states:

"We certainly question whether an EIS is desirable or necessary in this case. We also question whether a programmatic EIS should be required in any case where an adequate EA is provided. By definition, a programmatic EIS is concerned with broad issues rather than with specific considerations which relate to a particular site. In these experiments and, it seems to us in all but the most unusual circumstances, an EA will serve as well as an EIS to identify environmental effects. The EA is more concerned than the EIS with the present.

"To suggest that a programmatic environmental impact statement be developed for recombinant DNA technology after two or three experiments involving field tests of recombinant DNA organisms have been described is at best premature. It is a bit like asking

for description of the long-term effects of the discovery of x-ray on our civilization immediately after Rontgen showed the invisible radiation would penetrate some objects and not others and that these effects could be measured. Science deals with limited extrapolation from known experimental results; the data from one experiment must be digested and interpreted before the next can begin."

III-B-1-j. National Agricultural Chemicals Association

The letter from Dr. Spurrier states:

"From our point of view, the Environmental Protection Agency and the U.S. Department of Agriculture, including APHIS, are taking leadership roles in agriculturally oriented research dealing with recombinant DNA and genetically engineered plant varieties. With the already published EPA interim guidelines dealing with pesticide research and notification, and the soon-to-be proposed rulemaking on the subject, it appears that the role of NIH, as oversight, should in the future be directed to only those activities of broad national concern involving public health issues. The technology of bio/genetic engineering, particularly in agriculture, is progressing in a very positive manner. The need for detached oversight such as the development of very comprehensive Environmental Impact Statement by NIH to comply with certain interpreted requirements of NEPA seems now to be procedurally redundant unless there is a scientific reason to question a basically unknown revelation.

"At this time, there is no reason to believe that genetically engineered organisms are any different than organisms that mutate during natural selection. Over the years, plant breeding techniques have used chemically induced mutations to assist in mass selection. In genetic engineering, the biological derivation is actually more precise and predictable. It would appear that very strong arguments would have to be made to justify the need for a programmatic Environmental Impact Statement to demonstrate that a genetically engineered organism has a greater chance to invoke environmental harm than an organism altered by traditional practice."

III-B-2. Comment Indicating the Need for a Programmatic EIS "Is Unlikely to Be Resolved in the Near Future."

The letter from Dr. Suslow states:

"The need for a programmatic EIS to address any release of genetically engineered organism is unlikely to be resolved in the near future."

Dr. Suslow then goes on to give arguments which seem to support the concept that a programmatic EIS is not required when he states:

"Environmental concerns and risks must be expressed on a case-by-case basis (as was the original approach of NIH-RAC) due to the large

degree of variability in organism, target site, and introduced or altered gene function. I don't believe anyone can predict the diversity of potential basic or applied research needs that would cover all situations or sources of risk."

However, while Dr. Suslow does not seem to favor a single programmatic EIS for all NIH approvals of proposals to release into the environment organisms containing recombinant DNA, he then goes on to argue for "programmatic statements" of "broad groupings of potential releases." He states:

"I do believe programmatic statements are sorely needed to define the expected environmental fitness of broad groupings of engineered organisms and the potential risk of detectable persistance. It would be against this series of broad statements compiling currently available information that lay people and cross-disciplinary scientists could make preliminary judgements concerning risk to the environment. For example, where the general need or intent of a research program is to release and test a biological control agent, the organism must be environmentally competent and competitive. This would be covered by different broad criteria than an engineered Rhizobium which can be introduced to plants in a way that avoids its competitive disadvantage to other native Rhizobium spp and can thus be tested, at a preliminary stage, without concern for its ability to persist. Similar examples can be drawn for differences between deletion mutations and novel expression or novel regulations of genes in the released organism, etc. Thus by compiling data to cover broad groupings of potential releases by, perhaps, experimental objective as it relates to environmental fitness and subdivided by function (i.e. toxin, antibiotic, enzyme, polysaccharide, etc.) guidelines covering experimental requirements prior to release might be more readily determined. Hopefully this could expedite consideration and review of release proposals by highlighting potential areas of concern for the researcher. More detailed analysis of the individual experiment could then refer to a more generalized environmental statement if the review committees find no significant risk in the proposed release. No additional comprehensive or site-specfic EIS would be needed for small scale testing."

III-B-3. Comment Indicating a Programmatic EIS is Required

The only comment received indicating that a programmatic EIS is required for NIH approvals of release into the environment of organisms containing recombinant DNA molecules was the following sentence from Mr. Rogers' May 14 letter:

"For the reasons stated in our appellate brief, which arguments we incorporate by this reference, and as indicated by the court of appeals opinion on the question, there are a number of factors suggesting that a programmatic EIS is required."

III-B-4. My Response to Comments on Requirement for Programmatic EIS

As indicated in Section III-B-3, just above, Mr. Rogers wrote one sentence indicating that a programmatic EIS is required. That sentence refers to "the court of appeals opinion...suggesting that a programmatic EIS is required." In fact as noted in the April 15, 1985, Federal Register notice, the Appeals Court reversed "the District Court's finding that plaintiffs are likely to succeed in showing, at this point, that the governing law requires NIH to complete a programmatic EIS on deliberate release experiments."

While one sentence of comment was received indicating that a programmatic EIS is required, on the other hand, numerous, detailed, cogent arguments were advanced in ten different letters (which are given in Section III-B-1 of this memorandum, above) indicating why a programmatic EIS is not required in connection with NIH approvals of proposals to release into the environment organisms containing recombinant DNA. I recommend that you find that such a programmatic EIS is not needed at this time. However, if the NIH role with respect to deliberate release experiments changes in the future, we can reevaluate the need for a programmatic EIS at that time.

III-C. Comments and Response to Comments on Adequacy of April 15, 1985, Federal Register Notice

III-C-1. Mr. Rogers in his May 14 letter states:

> "As a preliminary matter, we wish to point out a significant omission in the notice in its description of the opinion of the Court of Appeals in FET v. Heckler. The court of appeals agreed with the district court that the National Environmental Policy Act (NEPA) applies to the deliberate release experiments, the court stating 'that if NIH fails to give appropriate environmental consideration to any other experiment, as it has failed to do with the University of California experiment, injunctive relief would be clearly proper.' Slip op. at 4: see also Slip op. at 31.
>
> "The difficulty with the notice on this point is that it is consistent with -- and therefore could be erroneously construed to mean -- that the court held only that NEPA applies to the University of California experiment. In light of the importance of the applicability of NEPA to all deliberate release experiments conducted by entities receiving recombinant DNA funding from NIH, a corrective public notice should be issued on this point."

III-C-2. My Response to Comments in Section III-C-1

I do not believe the April 15, 1985, Federal Register notice is misleading. The notice did not purport to give a complete analysis of the Court of Appeals decision, and the quotations from and references to the decision in the notice are accurate. A complete discussion of the details of the

decision would note have been consistent with the purpose of the notice and was not necessary since the complete text of the decision is available to the public. Furthermore, the notice specifically states that "the Appeals Court went on to state that, 'NIH should at least consider whether a programmatic EIS is required to fulfill the agency's legal obligations under NEPA and the CEQ regulations.'" This clearly indicates in the notice, that the Court did not hold that NEPA applies only to the University of California experiment. I, therefore, do not see any reason to issue "a corrective public notice," and I recommend you not accept this suggestion.

III-C-3. Mr. Rogers in his May 14 letter states:

> "Turning to the question of whether a programmatic EIS is necessary for the NIH activities involved in the review and the decisions on such experiments, we wish to state the public notice discussion of the issue is totally inadequate. The notice fails to reflect an objective and even handed approach to the question. The court of appeals made it abundantly clear that the NIH was under an obligation to undertake a 'reasoned consideration' of the factors relevant to resolving that issue. Slip op. at 32. 'Since NIH has given no serious consideration to whether a programmatic EIS is justified, we cannot evaluate its claims that deliberate release experiments are neither so 'cumulative' or 'connected' that a programmatic EIS is required under the CEQ regulations, nor so 'similar' that a programmatic EIS may not be 'the best way to assess adequately' their environmental effects. 40 C.F.R. [Sec.] 1508.25.
>
> "The public notice reflects a NIH's continued refusal to give serious consideration to the issue. The discussion of that issue consists primarily of a cryptic reiteration of the arguments made in court against the notion that the NIH is required to prepare such a programmatic EIS, even though most of those arguments would be equally applicable to containment experiments, the guidelines for which prompted NIH to prepare an EIS....
>
> "The public will not be adequately informed of the issues involved on the questions on whether a programmatic EIS is necessary unless there is at least a reasonable good faith attempt to set forth both sides of the issue in a public notice on the question. Accordingly, we request NIH to vacate the notice and issue an adequate one."

III-C-4. <u>My Response to Comments in Section III-C-3</u>

I do not believe the public notice is inadequate or that it "fails to reflect an objective and even handled approach to the question." It objectively and fairly gives factual information to the public in the following four areas:

A. When is a Programmatic EIS Required?

B. Field Tests Approved by NIH.

C. NIH's Role in the Approval or Disapproval of Proposals for the Release into the Environment of Organisms Containing Recombinant DNA.

D. NIH's Future Role in the Review and Approval of Proposals for the Deliberate Release Into the Environmental of Organisms Containing Recombinant DNA.

Mr. Rogers does not cite any additional facts that he believes should have been included in the notice.

It should also be noted that there was no requirement that the NIH solicit public comment on the issue of whether or not a programmatic EIS is required. That the NIH solicited public comment through a Federal Register notice was an act on the NIH's part that went beyond any requirements of the National Environmental Policy Act (NEPA), or the CEQ Regulations Implementing NEPA, or the HHS General Administration Manual, or the decision of the Court of Appeals. Thus, contrary to Mr. Rogers' assertion, I believe the notice indicated NIH's intention to give the issue serious consideration. I do not see any reason "to vacate the notice," and issue a new one, and recommend that you not accept this suggestion.

IV. Summary of Recommendations

This section of the memorandum recapitulates the recommendations which were made earlier in the memorandum, and provides a place for you to indicate whether or not you concur or do not concur with each recommendation.

IV-A. In Section III-A-3-a-(2) of this memorandum, I consider six comments on the January 21, 1985, EA-FONSI, submitted by Dr. Anne K. Vidaver. I recommend accepting five changes in EA-FONSI, and that no change be made in response to the sixth comment.

Concur

Do Not Concur

7-2-85
Date

IV-B. In Section III-A-3-b-(2) of this memorandum, I consider comments on the EA-FONSI submitted by Dr. David Pimentel. In response to his comments, I recommend changes in the EA-FONSI.

James B. Wyngaarden
Concur

Do Not Concur

7-2-85
Date

IV-C. In Section III-A-3-b-(4) of this memorandum, I consider comments on the EA-FONSI submitted by Mr. Rogers. I recommend that no change in the EA-FONSI be made in response to these comments.

James B. Wyngaarden
Concur

Do Not Concur

7-2-85
Date

IV-D. In Section III-A-3-b-(6) of this memorandum, I consider comments submitted by Drs. Pimentel and Gliessman. In response to these comments, I recommend addition of text to the EA-FONSI.

James B. Wyngaarden
Concur

Do Not Concur

7-2-85
Date

IV-E. In Section III-A-3-b-(8) of this memorandum, I consider comments submitted by Dr. Pimentel and Gliessman. I recommend that no change be made in response to these comments.

James B. Wyngaarden
Concur

Do Not Concur

7-2-85
Date

IV-F. In Section III-A-3-b-(10) of this memorandum, I consider comments submitted by Dr. Rogers and Dr. Gliessman. I recommend that no further action be taken in response to these comments.

J B Wyngaarden
Concur

Do Not Concur

7-2-85
Date

IV-G. In Section III-A-3-b-(12) of this memorandum, I consider comments submitted by Mr. Rogers. I recommend that you reaffirm the adequacy of the Environmental Assessment that you signed on January 21, 1985.

J B Wyngaarden
Concur

Do Not Concur

7-2-85
Date

I recommend that you reaffirm the Finding of No Significant Impact that you made on January 21, 1985.

J B Wyngaarden
Concur

Do Not Concur

7-2-85
Date

I recommend that you reject the suggestion that the NIH should withdraw the EA-FONSI and should prepare an EIS on the experiment.

J B Wyngaarden
Concur

Do Not Concur

7-2-85
Date

I recommend, based on the Environmental Assessment and Finding of No Significant Impact, that you indicate your intention, once the Court lifts the injuction prohibiting NIH from reapproving the Lindow-Panopoulos field test, to reapprove the field test under the alternative discussed in Section 4.2.3 of the January 21, 1985, EA-FONSI, with the even more conservative proviso that the field test will be approved only for Pseudomonas syringae, and not to include Erwinia herbicola.

J B Wyngaarden
Concur

Do Not Concur

7-2-85
Date

IV-H. In Section III-B-4 of this memorandum, I consider comments regarding the need for a programmatic Environmental Impact Statement (EIS) in connection with NIH approvals of proposals to release into the environment organisms containing recombinant DNA. I recommend that you find that such a programmatic EIS is not needed at this time.

J B Wyngaarden
Concur

Do Not Concur

7-2-85
Date

IV-I. In Section III-C-2 of this memorandum, I consider comments submitted by Mr. Rogers concerning adequacy of the April 15, 1985, Federal Register notice. I recommend that you reject Mr. Rogers' request for "a corrective public notice."

J B Wyngaarden
Concur

Do Not Concur

7-2-85
Date

IV-J. In Section III-C-4 of this memorandum, I consider other comments submitted by Mr. Rogers concerning the adequacy of the April 15, 1985, Federal Register notice. I recommend that you reject Mr. Rogers' request that NIH vacate the notice and reject his request that NIH issue a new notice.

Concur	Do Not Concur	7-2-85 Date

V. Future Action

If you concur with all the recommendations in Section IV of this memorandum then: a notice will be placed in the Federal Register indicating your decisions and the availability to the public of copies of this memorandum; and we will ask the District Court to lift the preliminary injunction on the Lindow-Panopoulos field test.

ENVIRONMENTAL ASSESSMENT
AND
FINDING OF NO SIGNIFICANT IMPACT

Concerning the application to the National Institutes of Health (NIH) under the NIH Guidelines for Research Involving Recombinant DNA Molecules by Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damage to plants.

Prepared by the
National Institutes of Health
Public Health Service
U.S. Department of Health and Human Services

Concurrence: James B Wyngaarden
James B. Wyngaarden, M.D.
Director
National Institutes of Health
Date: 1-21-85

Table of Contents

		Page
1.0	Introduction and Summary	1
2.0	Background: Previous Administrative Action	3
3.0	Purpose and Need	8
3.1	Purpose of the Proposed Action	8
3.2	Need for the Proposed Action	9
4.0	Description of Proposed Action and Alternatives	11
4.1	Proposed Action	11
4.2	Alternatives to the Proposed Action	16
4.2.1	Approval of Original Proposal (September 17, 1982) Without Restriction	16
4.2.2	Approval of Modified Proposal (March 8, 1983) Without Restriction	16
4.2.3	Approval of Proposal as Approved by NIH on June 1, 1983, With Changes Proposed by the Investigators	16
4.2.4	Withhold Approval	17
5.0	Environmental Effects	17
5.1	The Physical Environment	17
5.2	Proposed Test Organisms	22
5.2.1	Characteristics of *P. s. syringae* and *E. herbicola* strains	22
5.2.2	Construction of INA Deletion Mutants	28
5.2.3	Previous Field Tests with INA Minus Bacteria	29
5.3	Effects of the Proposed Field Test	30
5.3.1	Risk Assessment	30
5.3.2	The Test Plot	34
5.3.3	Crops and Non-Commercial Plant Species	36
5.3.4	Local Epiphytic Bacterial Populations	37
5.3.5	Insect Populations	39
5.3.6	Animal Populations	41
5.3.7	Atmosphere and Climate	41
5.3.8	Scientific and Agricultural Benefits	45
5.3.9	Worst Case Considerations	46

5.4 Effects of Alternatives 48
5.4.1 Approval of Original Proposal Without Restriction. . . 48
5.4.2 Approval of Modified Proposal Without Restriction. . . 49
5.4.3 Approval of Proposal as Approved by NIH on June 1, 1983, With Changes Proposed by the Investigators. 49
5.4.4 Withhold Approval. 50

5.5 Summary and Conclusions 50

6.0 Consultation 52

7.0 References 53

Appendix A Background Concerning the NIH Guidelines for Research Involving Recombinant DNA Molecules 57

Appendix B Description and Location of Tulelake Field Station. B1

Appendix C Diagram of Test Plot and Description of Treatments. C1

1.0 Introduction and Summary

This document is an Environmental Assessment (EA) and Finding Of No Significant Impact (FONSI) concerning the application, under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (Guidelines), by Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test certain strains of ice-nucleation-minus bacteria prepared by recombinant DNA techniques to determine whether those bacteria prevent frost damage to plants. The finding of no significant impact confirms the finding that the proposed field test presented "no significant risk to health or the environment" which NIH previously made under the NIH Guidelines and published in the Federal Register. (A)

References cited in this document are tabulated in Section 7.0 of this document. They are available for public inspection at the NIH Office of Recombinant DNA Activities (ORDA), Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland 20205, on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

This Environmental Assessment and Finding Of No Significant Impact has been prepared in accordance with: (1) the National Environmental Policy Act of 1969, as amended (B); (2) 40 CFR §1501.3(b) and §1508.9(b) of the Council on Environmental Quality Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act (C); and (3) Part 30, Environmental Protection, of the General Administration Manual of the Department of Health and Human Services (D). Authority to approve such an EA and FONSI was delegated to the Director, NIH, by the Director, Office of Management, United

States Public Health Service, in his memorandum to PHS Agency Heads, dated June 2, 1980, on the subject, "Delegation of Authority for Carrying Out Responsibilities Under Revised Part 30 of the General Administration Manual, Environmental Protection."

The proposed field test to be conducted on a small plot at the Tulelake Research Station in Tulelake, California, would involve bacteria, common plant epiphytes, made "ice-nucleation-minus" (INA^-) by recombinant DNA techniques. The bacteria would be sprayed at different times on sixteen 40-foot rows of potato, tomato and snapbean plants, to see if they protect the plants from frost damage.

The NIH has assessed the environmental effect of the proposed field test and has found that it will not have a significant impact on the environment for the following reasons:

1. The INA bacteria prepared by recombinant DNA techniques will not express any foreign proteins and neither the original nor the altered bacteria are pathogenic to living creatures (insects, animals, birds, man). The original and altered bacteria to be used in the experiment have been shown not to be pathogenic to any of the major crops growing in the area.

2. Although small numbers of the INA^- bacteria may be carried away from the test site by insects, birds and the wind, no environmental harm will result from this, because:

(a) In order to survive and multiply, the genetically engineered INA^- bacteria would have to compete successfully against much larger numbers of INA^+ bacteria and naturally occuring INA^- bacteria and other bacteria. Established natural populations of bacteria on surrounding plants will retard the INA^- bacteria from colonizing or becoming established on neighboring plants.

(b) Even if many of the genetically engineered bacteria survived and multiplied, no significant harm would result since INA^- bacteria already exist in nature with no harmful effects and chemically altered INA^- bacteria have previously been released with no adverse environmental effects.

3. The large seasonal fluctuation and widely varying relative abundance of INA^+ bacteria versus INA^- bacteria in nature, as well as the large reductions of INA^+ bacteria from traditional agricultural practices, have all occurred without reported environmental impacts. The potential slight, localized and transitory increase in the number of INA^- bacteria resulting from the proposed field test will not result in any environmental impact of significance beyond the test plot on either a local, regional or global scale.

2.0 Background: Previous Administrative Action

Under Section III-A-2 of the Guidelines, (a background statement on the Guidelines is attached as Appendix A) an experiment involving release

into the environment of any microorganism containing recombinant DNA cannot be initiated without submission of relevant information on the experiment to NIH, the publication of the proposal in the Federal Register for 30 days of comment, review by the NIH Recombinant DNA Advisory Committee (RAC) and a specific approval by NIH based on its finding that the experiment will present "no significant risk to health or the environment."

On September 17, 1982, Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, requested permission from NIH to field test Pseudomonas syringae pv. syringae and Erwinia herbicola carrying in vitro generated deletions of all or part of the genes involved in ice nucleation for purposes of biological control of frost damage to plants. (E)

On September 22, 1982, NIH gave notice of this request in the Federal Register announcing that it would be considered at the October 25, 1982, RAC meeting and that "interested parties are invited to submit comments." (F)

In addition to publication in the Federal Register, this notice was sent to more than 3,000 interested persons who, at their request, are on a mailing list to receive Federal Register notices related to the NIH Guidelines for Research Involving Recombinant DNA Molecules. Furthermore, all RAC members, including the liaison members to the RAC from other Federal agencies, and others, were sent, prior to the October 25, 1982, RAC meeting, a copy of the Federal Register notice and a copy of the Lindow-Panopoulos, 98 page, September 17, 1982, submission. Copies were also made available to all members of the public present at the October 25, 1982, RAC meeting.

No public comments on the Lindow-Panopoulos submission were received in response to the September 22, 1982, Federal Register notice. The Lindow-Panopoulos submission was reviewed at the October 25, 1982, RAC meeting attended by 16 RAC members, 23 representatives of Federal agencies other than NIH, and 43 others. The RAC recommended approval of the Lindow-Panopoulos proposal by a vote of seven in favor, five opposed, and two abstentions. (G)

In the Federal Register of January 10, 1983, NIH announced that "because of concerns raised at the RAC meeting, approval of the proposed field tests is being withheld. The investigators may bring this or a modified proposal back for consideration at a future RAC meeting." (H)

On March 8, 1983, Drs. Lindow and Panopoulos submitted a revised proposal (I). This proposal was modified compared to the September 17, 1982, proposal, in response to environmental concerns raised at the October 25, 1982, RAC meeting. Among other changes it provided for testing in only one small field rather than at the six locations proposed in the original request, it stated that only chromosomal antibiotic resistance markers would be used to monitor the strains, and it addressed the concern regarding the possible effect of the field test on rainfall.

On March 4, 1983, NIH gave notice of this request in the Federal Register announcing that it would be considered at the April 11, 1983, RAC meeting and that "interested parties are invited to submit comments." (J)

In addition to this notice being distributed to all those who receive the Federal Register, it was again sent by the NIH Office of Recombinant DNA

Activities (ORDA) to its mailing list of more than 3,000 individuals. All RAC members, including the liaison members to the RAC from other Federal agencies, were sent, prior to the April 11, 1983, RAC meeting, a copy of the March 4, 1983, Federal Register notice and a copy of the Lindow-Panopoulos, 80 page, March 8, 1983, revised proposal. Copies were also made available to all members of the public present at the April 11, 1983, RAC meeting. No comments on the Lindow-Panopoulos revised submission were received by NIH in response to the March 4, 1983, Federal Register notice.

The revised Lindow-Panopoulos submission was reviewed at the April 11, 1983, RAC meeting. Present for all or part of the meeting were 23 RAC members, 17 representatives of Federal agencies other than NIH, and 49 others.

At its April 11, 1983, meeting the RAC found that the proposal had been modified to address the environmental concerns raised at the October 25, 1982, meeting. In addition, the new proposal contained a discussion of biosafety considerations which went beyond that contained in the original proposal. The RAC found that organisms modified by classical genetic techniques had already been released with no harm. Nevertheless, the RAC did insist that the procedures for elimination of the genetically engineered INA$^-$ bacteria (in the unlikely event the plants were harmed) be modified such that no antibiotics be used. With this modification the RAC recommended approval of the proposal, by a vote of 19 in favor, none opposed and no abstentions. (K)

The proposal was forwarded to the U.S. Department of Agriculture (USDA) which asked a consultant in the Department of Plant Pathology at the Ohio State University, Ohio Agricultural Research and Development Center to review the proposal.

He wrote (L), "I have read the request to the RAC from Drs. Steven Lindow and Nickolas Panopoulos which you sent me for comment. I find their proposal to field test ice nucleation deficient mutants of P. s. syringae and E. herbicola for biocontrol of frost injury to be a very worthwhile project which should be done. I cannot see that there is any hazard in constructing and releasing these mutants. Mere deletion of INA genes should not increase the fitness of these bacteria or what pathogenicity they might have. Furthermore, the use of rDNA procedures is designed to insure well-defined homogenic strains for experiments and should not generate any strains which could not otherwise arise by natural genetic processes. Drs. Lindow and Panopoulos appear to have thought of almost every possible means to minimize and monitor the spread of their bacteria in the environment. Moreover, even if the mutant strains are disseminated, it is doubtful that they would be able to establish new epiphytic populations from small doses of inoculum because resident populations of very similar bacteria are undoubtedly already established in their plots and surrounding crops." Subsequently, the USDA Recombinant DNA Committee recommended that NIH approve the proposed field test.

In the Federal Register of June 1, 1983, NIH announced that "permission is granted to Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to release under specified conditions Pseudomonas syringae pv. syringae and Erwinia herbicola carrying in vitro generated deletions of all or part of the genes involved in ice nucleation," along with the finding that this action presents "no significant risk to health or the environment. (A)

On September 14, 1983, a lawsuit against NIH was filed in the United States District Court for the District of Columbia. (M) The suit claimed that NIH had approved experiments involving release of recombinant DNA organisms into the environment without the preparation of an Environmental Assessment and/or an Environmental Impact Statement, in violation of the National Environmental Policy Act (NEPA) and the CEQ NEPA Regulations.

On May 16, 1984, the Court enjoined the NIH from "approving or continuing to approve experimentation involving the deliberate release of recombinant DNA under ... the ... NIH Guidelines ... until such time as the Court enters final judgment." The University of California was enjoined from proceeding with the Lindow-Panopoulos frost damage experiment approved by NIH on June 1, 1983, until such time as the Court enters final judgment. In its decision, the Court stated, among other things, that the NIH decision "not to support the very first deliberate release experiment with even an environmental assessment, ... strongly suggests the absence of a hard look inquiry." (N)

In response to the Court's decision, NIH has prepared this EA and FONSI.

3.0 Purpose and Need

3.1 Purpose of the Proposed Action

The purpose of the proposed field test is to determine the degree of protection from frost damage which the recombinant DNA-modified bacterial strain will afford to potato, tomato and bean plants under the prevailing conditions at the test site. The purpose of NIH approval of this proposed

field test is, as set forth in the Guidelines, to determine that the test complies with the Guidelines and presents no significant risk to health or the environment.

3.2 Need for the Proposed Action

Classical methods of frost control have many problems. Sprinkler irrigation of leaves for frost control requires large amounts of water and is ineffective when wind or poor sprinkler coverage prevents continuous wetting of the plants. The other methods (heating, mixing air layers, furrow irrigation) require large amounts of energy and water and are rapidly becoming prohibitively expensive. Artificially generated fogs can create safety hazards, and burning large quantities of fossil fuels can adversely affect environmental quality. (0)

P. syringae and E. herbicola, the so-called "ice-nucleation bacteria," normally live on plant leaf surfaces. Several scientists have found that these bacteria nucleate formation of ice crystals on the leaves when the temperature drops a few degrees below freezing. The ice crystals damage the plant. Such frost damage is a significant problem for fruit and vegetable growers and has a direct impact on the prices that consumers pay for these foods. If the bacteria are absent or if the very same bacteria having defects in the genes responsible for ice nucleation (which are called the "ice-nucleation" genes) are present, ice crystal formation occurs at a lower temperature. This means that the plants can survive colder temperatures without frost damage. Thus, if the normal bacterial population of the leaves could be replaced by bacteria that lacked the "ice-nucleation" genes, frost

damage could be reduced. The desired kind of altered bacterium can be generated by traditional methods that are used throughout the food and pharmaceutical industry to derive bacteria with desired characteristics. The disadvantage of traditional methods is that they are not very specific and often change an organism in both the desired way and, as a side effect, in other ways that can adversely affect the organism's survival ability or other characteristics of the organism. Recombinant DNA techniques offer, for the first time, a method for altering only the gene or genes that are desired to be altered. This is done by isolating the gene to be altered, changing it, and replacing the organism's original gene with the specifically altered gene. Thus the extent to which the organism is changed is minimized through the use of recombinant DNA techniques. As an important consequence, the probability that the altered organism will cause unexpected ecological problems is significantly reduced when recombinant DNA techniques are used. (P)

Field tests are essential to determine whether or not the introduced property, i.e., INA^- has any value, because it is impossible to simulate field conditions in the growth chamber or greenhouse. The researchers have conducted extensive experiments in the greenhouse and growth chamber, but can finally determine the efficacy of the INA^- bacteria only under field conditions. Based on previous experiments with chemically-induced and ultraviolet-radiation-induced INA^- mutants and naturally occurring INA^- Pseudomonas strains, the proposed field trials are expected to have a positive outcome, i.e., the INA^- strains are expected to protect the plants against frost injury. However, the degree of such protection varies from strain to strain as well as with environmental variables, both physical and biological. The physical variables of the environment include, among other things, soil, moisture, air humidity,

radiation intensity, including ultraviolet light, and diurnal fluctuation in temperature. The biological variables of the environment include the diversity of INA^+ bacteria and other biological agents which continually arrive at and colonize leaf surfaces. The physical and biological variables cannot be precisely duplicated in a greenhouse or growth chamber and, therefore, their collective effect can only be determined by field testing. There is no substitute for field tests, a time-honored method for testing the efficacy of living agents on plants. Such field tests are required to advance knowledge. Without such tests, the new technology cannot be used to protect plants from frost damage, precluding realization of the anticipated substantial economic benefits.

The need for NIH review and approval or disapproval of this proposed field test is to assure that there is review at the national level of whether the test can be conducted safely with no significant risk to health or the environment.

4.0 Description of Proposed Action and Alternatives

4.1 Proposed Action

For purposes of this Environmental Assessment, NIH proposes to reaffirm its June 1, 1983, approval for Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test under specified conditions bacteria with *in vitro* generated deletions of all or part of the genes involved in ice nucleation. The prior action was based on the NIH

determination that the field test, with the specified conditions, presented no significant risk to health or the environment.

In the initial field test, 16 rows of potato plants covering 0.4 acre at the Tulelake Research Station will be used in testing. 0.09 acre will be sprayed with suspensions (ca. 10^7 - 10^8 cells/ml) of genetically engineered ice-nucleation-negative deletion mutants of Pseudomonas syringae pv. syringae and Erwinia herbicola to test the suitability of the deletion mutants as frost damage biocontrol agents. Another 0.09 acre of potato plants will be sprayed with the same suspension, followed by application of a suspension of unmodified INA^+ bacteria of the same strains. Parallel experiments on the same plot will be conducted with chemically-induced INA^- mutants. For comparison, another 0.044 acre of potatoes will be treated with only INA^+ bacteria, and a similar plot will be left untreated as a control (see complete description in Section 5.3.2). Field tests will be conducted on a continuing basis during the spring and fall of the test period, when frost is imminent. Subsequent experiments will involve the same strains of bacteria and the same experimental procedures, except that tomatoes and beans will also be used as the target crop.

The bacteria are common plant epiphytes found in substantial numbers on above-ground plant surfaces (leaves, twigs, buds, flowers) with seasonal fluctuation from undetectable levels up to 10^5 cells/gram tissue fresh weight. A causal relationship between frost damage on frost-sensitive crop plants at relatively warm subzero temperatures (down to -5° C) has been established through experiments showing that the degree of damage, after exposure to low temperatures (either in the field or in growth chambers), is directly related to the populations of ice-nucleation-active (INA^+) bacteria present in, or on

the surfaces of, above-ground plant parts. It has also been shown that plants grown aseptically can tolerate temperatures of several degrees Celsius below zero without apparent damage and they are rendered sensitive to such temperatures by spraying with suspensions of INA^+ bacteria prior to the low temperature exposure. (I)

Frost damage to plants can be decreased by reducing the natural population of INA^+ bacteria present on the plant surfaces. Especially suited for this purpose are INA^- mutants of the naturally occurring INA^+ strains. These INA^- mutants, being basically adapted for epiphytic growth and survival, would presumably prevent their wild-type counterparts from occupying the same physical spaces on the plant surface and from using the same nutrients. (I)

INA^- deletion mutants of selected strains of P. syringae pv. syringae and E. herbicola will be constructed by Drs. Lindow and Panopoulos in their laboratories in Rooms 210, 216, 225 and 227 Hilgard Hall on the Berkeley campus. A biosafety cabinet is located in Room 210. INA^- deletions will be obtained by removing one or more DNA fragments covering all or part of the INA region. Recombinant plasmid DNA will be digested with restriction endonuclease(s) which cleave the cloned inserts at suitable sites and the DNAs will be religated and transformed into E. coli. INA^- transformants will be verified as having specific fragment deletions by plasmid reisolation, restriction endonuclease re-cutting and/or blot hybridization.

To facilitate the monitoring of the mutant INA bacteria, various types of "flagged" deletions, having specific DNA sequences or segments, are proposed to be constructed. Elements or segments encoding for resistance to antibiotics

used in plant disease control will not be used. The DNA sequences or segments flagging the deletions provide a means for positive identification of strains carrying the deletions by dot-blot hybridization with the respective DNA probe. This makes possible the large-scale monitoring of bacterial populations on a routine basis to determine survival and lateral dissemination of strains. (I)

Prior to field application, the mutant strains were tested in a growth chamber and/or greenhouse, to verify that they did not induce frost or other injury to plants and were not capable of colonizing their leaves to a greater extent than the original strain. These preliminary contained experiments provided data on the ability of the mutant INA^- bacteria to displace wild strains from leaf surfaces. In the growth chamber and greenhouse the plants were sprayed with suspensions of INA^- deletion mutants. The plants were then challenge-sprayed with INA^+ parental or heterologous strains or allowed to be naturally colonized with wild INA^+ strains. Frost injury was assessed for plants sprayed with either or both types of bacteria as well as for nonsprayed controls after growth chamber exposure to subzero temperatures. (Z)

Survival, lateral dissemination, and population dynamics of INA^- mutants and INA^+ populations in the field and the specificity if any, of INA^- and INA^+ bacterial interactions on leaf surfaces will be studied in parallel. To facilitate these studies, the strains will be marked with antibiotic resistance mutations not used in clinical medicine so that they can be selectively reisolated. Measurements of the applied bacteria will be made at 10m, 100m and 1km in all directions from the site of application. Measurement of bacteria carrying the appropriate genetic marker will be made before treatment and weekly for the first month after treatment. Thereafter, treatment will be at

monthly intervals. If appropriately marked strains are not detected by standard dilution plating procedures these procedures will be repeated following standard enrichment procedures. (I)

The total number of bacteria released in each application season (fall and spring) would not exceed 10 liters of fully grown cultures. It is estimated that 10 liters of culture would contain approximately 5×10^{12} cells. Bacterial suspensions applied to plants will contain 10^7 - 10^8 cells/ml or 10^{10} - 10^{11} cells/liter. The quantity of suspensions applied in the field will be 20 liters/acre. Only 0.09 acre is needed for each trial of a INA^- deletion strain. Since P. s. syringae is ubiquitous, the numbers of bacteria released in the experiment would be infinitesimal compared to the numbers of P. syringae occurring naturally. (I)

Application will be by hand spraying using a conventional sprayer or by dipping the tubers in a powder. To prevent inhalation of bacterial aerosols respirators will be used. The bacteria will be applied to plants not in the immediate vicinity of other similar plant species to minimize the chance of significant spread of the bacteria out of the treatment area during application. Plant parts will not be transported away from the site of application. Bacterial cultures will be transported from the Berkeley campus to the field station in sealed, leakproof containers. Dilutions to a larger volume will be made on site. (I)

Although both the original (September 17, 1982) and amended (March 8, 1983) proposals called for the use of bactericides to eliminate the suspect organisms in the unlikely event that the plants developed any injury

attributable to the modified bacteria, the NIH Recombinant DNA Advisory Committee (RAC) rejected this aspect of the proposal and recommended that NIH approve the proposal only if bactericides were not used and if all above ground plant parts are burned or preferably buried at the test site.

4.2 Alternatives to the Proposed Action

4.2.1 Approval of Original Proposal (September 17, 1982) Without Restriction

Under this alternative NIH would be approving the field tests described in section 4.1 except that: the tests would be conducted at six sites instead of one; and antibiotics would be sprayed on the plants to kill the bacteria in the unlikely event the plants suffer injury attributable to the modified bacteria or the modified bacteria are detected in significant numbers beyond the test plot.

4.2.2 Approval of Modified Proposal (March 8, 1983) Without Restriction

Under this alternative the field test would be the same as that described in section 4.1 except that antibiotics would be sprayed on the plants to kill the bacteria in the unlikely event the plants suffer injury attributable to the modified bacteria or the modified bacteria are detected in significant numbers beyond the test plot.

4.2.3 Approval of Proposal as Approved by NIH on June 1, 1983 With Changes Proposed by the Investigators

Under this alternative the field test would be the same as that approved by NIH on June 1, 1983 except that the following restrictions subsequently proposed by Dr. Lindow (Y) would be incorporated: (1) flagged deletions having specific DNA sequences or segments physically linked to deletions in the ice nucleation gene would not be used to tag the modified INA⁻ bacteria for purposes of monitoring those bacteria for at least the first year of the field tests; and (2) ice nucleation deficient mutants of Erwinia herbicola will not be used for at least the first year of the field test.

4.2.4 Withhold Approval

Under this alternative NIH would reverse its previous approval of the field test.

5.0 Environmental Effects

5.1 The Physical Environment

The proposed test site for the experiment is a portion of the Tulelake Research Station, located approximately two miles southeast of Tulelake California. (See Appendix B for maps and further description.) The test plots occupy approximately one acre on the 100-acre section of the field station and will be buffered by 20 feet of fallow land and a ten-foot road on all sides. In addition, there will be a band of untreated plants around the edge of the 0.4 acre plots to protect the treated plants.

The Tulelake Research Station is located at the approximate center of the Tulelake Basin. The basin is high desert, irrigated and heavily agricultural. Irrigation is provided by the Tulelake Irrigation District, which serves over 90 percent of the land in the basin. Federal lands surrounding the basin are managed by the Bureau of Land Management and the U.S. Forest Service, and some of this land is leased for pasture. Livestock raised include cattle, sheep, and to a much lesser extent, hogs. Altogether, livestock is a very minor component of the agricultural use of the basin. The soil is predominantly sandy clay loam, rich in organic matter. The pH of the soil is in the range of 7.8, which indicates that it is alkaline.

Agricultural Use

The acreages presented in the table below are taken from the 1982 Annual Report of the Tulelake Irrigation District. The irrigation season generally starts in late April or May and extends through to mid- to late-September for some post-harvest irrigation.

Crops	Acres
Barley	7,600
Brew Barley	26,300
Feed Wheat	1,300
Durum Wheat	1,200
Oats	3,100
Peas	200
Rye	100
Alfalfa Hay	7,000
Other Hay	500
Pasture	1,800
Potatoes	11,000
Onions	2,000
Horseradish	700
Crop Subtotal	62,700

Other Land Uses	Acres
Idle Acres	56
Farmsteads, Road, and Drains	630
Residential, Commercial and Industrial	122

The 122 acres in residential, commercial, and industrial use includes a total of 118 acres for the Tulelake Research Station. In addition to the primary 18 acre site, the University of California leases a 100 acre plot from the Bureau of Land Management. The field test will be conducted on this plot.

Pesticide Usage

Farming practices in the area typically include the use of insecticides and fungicides. Insecticides are used for aphid control in potatoes, grain and alfalfa. Typical insecticides are parathion and a product trade-named "Monitor." Fungicides are used for early blight control, particularly in potatoes. They include copper-based and other fungicides. The normal application of these pesticides is by aerial crop dusting.

Evapotranspiration Rates

At Tulelake Station, the evapotranspiration rates are 0.06 inches of water per day in the spring and 0.25 inches per day during the summer. These figures include water loss though direct evaporation from the soil and through transpiration from the plants.

Temperatures and Wind

The following table presents monthly average maximum and average minimum temperatures, the number of days of frost, and average wind speeds for the months during which the field test would be conducted.

Months	Avg Max Temp (Degrees F)	Avg Min Temp (Degrees F)	Days of Frost	Avg Wind Speed (mph) (Day/Night)
Sept. 1983	76	35	9	2.6/2.3
Oct. 1983	66	29	23	2.0/1.3
Nov. 1983	41	26	21	3.2/2.3
	*	*	*	
Mar. 1984	52	24	28	4.5/2.6
Apr. 1984	51	27	28	5.0/2.8
May 1984	66	35	13	5.0/3.0
June 1984	71	39	6	4.0/3.0
July 1984	87	46	0	3.0/2.0
Aug 1984	86	41	0	3.0/2.0

Days of frost means those days with recorded overnight low temperatures of 32° F or less. At Tulelake, frost can occur any day of the year. Typical summer maximum daytime temperatures are in the range of 85° F. Prevailing winds blow from the southwest to the northeast.

Surrounding Animal and Human Populations

The 100-acre plot on which the field test will be conducted is approximately 2 miles southeast of the town of Tulelake, population 380. The plot is approximately 3 miles northwest of the town of Newell, and about 3½ miles from the Tulelake airport which is just outside (northeast) of Newell. Rural homes and small settlements are located throughout the area, particularly

along Highway 139 and the Southern Pacific Railroad right of way (adjacent to the highway), which are ½ to one mile northeast and east of the plot.

Tulelake Sump consists of two reservoirs which provide water storage for the irrigation system. One is located approximately 2½ miles west of the plot and the other is located 3½ miles south of the plot. The boundary of the Tulelake National Wildlife Refuge, which encompasses the Tulelake Sump, is about ½ mile south of the plot. Migratory waterfowl that use the Tulelake National Wildlife Refuge as a stopover during migrations through the Pacific flyway, include: Canada Goose, Snow Goose, White-Fronted Goose. Ducks: Pintail, Mallard, Widgeon, Green-Winged Teal, Cinnamon Teal, Gadwall, Shoveller, Scaup, Ring-Necked, Redhead, Canvasback, Bufflehead, Golden Eye. Mergansers: Redbreasted, Common, Hooded. Other waterfowl include Loons, Grebes, Cormorants, and White Pelicans. (S)

The fish population in the sump consists of Lamprey, Sturgeon, Trout, Lost River Suckers, Klamath, Large Scale Suckers, Catfish, Sunfish, Perch and Minnows. (S)

The south end of Tulelake Basin abuts the Lava Beds National Monument and sections of the Modoc National Forest. The former is located approximately five miles south of the plot and the latter about seven miles south of the plot. The forest consists of East Side Ponderosa Pine, Ponderosa Pine, White Fir and Red Cedar at the lower elevations, Shasta, Red Fir, and White Fir at the intermediate elevations and Lodge Pole Pine and Mountain Hemlock at the high elevations. Birds in the forest and in the basin include: Pheasant, Quail, Bald Eagle, Sage Grouse, Prairie Falcon, Owls, Pileated and Downy

Woodpeckers, Western Meadowlark, Evening Grosbeak and the Mountain Bluebird. Two hundred fifty-four species of birds have been observed in the surrounding area. Large and small mammals in the surrounding area include Deer, Elk, Bear, Bobcats, Pronghorn Antelope, Coyotes, Beavers, Badgers, Squirrels, Rabbits, Rats and Mice. (R) and (S)

5.2 Proposed Test Organisms

5.2.1 Characteristics of P. s. syringae and E. herbicola strains

A. P. s. syringae

1. Nomenclature and Taxonomy

P. s. syringae is an abbreviated taxonomic designation of Pseudomonas syringae pathovar (pv.) syringae currently accepted by the Judicial Committee of the International Committee on Systematic Bacteriology (the "pathovar" system) and is synonymous with the earlier designation Pseudomonas syringae. A large number of other plant pathogenic Pseudomonads distinct in their pathogenicity and biology from P. syringae pv. syringae are also classified as P. syringae in the current "pathovar" system but each has a different "pathovar" designation (e.g. P. syringae pv. phaseolicola earlier known as P. phaseolicola) reflecting their pathogenic specialization. (I)

2. Pathogenicity

P. s. syringae is a common plant epiphyte. It has never been shown to be a pathogen for humans or animals and does not grow at mammalian body temperature or at any temperature above 33-34° C. Neither P. s. syringae nor Erwinia herbicola is a pathogen of potato (See Index of Plant Diseases in the U.S., Crops Res Div, ARS, U.S.D.A., Aug. 1960; Compendium of Potato Diseases, W.J. Hocker ed., Amer. Phytopathol. Society, 1981; Diseases of Potato, G.J. Brencheley and H.J. Wilcox, Ministry and Agriculture, Fisheries and Food, London, U.K., 1979).

Certain strains or isolates of P. s. syringae have been reported to be pathogenic on many woody and herbaceous plants including bean, lima bean, wheat, barley, sorgum, corn, pear, apple, plum, peach, cherry, citrus, and others. However, disease caused by this pathogen on these hosts is rare in California and probably occurred only after predisposition by frost injury.

The pathogenicity of many isolates of pathogenic strains, obtained either from disease lesions on herbaceous hosts or from epiphytic inocula present on such hosts, is restricted to a few hosts which are closely related. This is true, for example, for isolates from bean and stone fruits. Other studies, however, indicate less well defined host specialization (broader or overlapping host range for individual isolates), and P. s. syringae has been considered by some to be a less specialized pathogen than otner P. syringae pathovars. However, there are several reasons to doubt many of the claims in the literature regarding its host range. For example, tests on host plants, in addition to or including the host of origin, were often conducted under conditions that were artificial in important respects:

(a) greenhouse instead of field conditions with prolonged moisture, non-fluctuating temperature, protection from UV light, etc.;

(b) heavy inoculum (typically 10^8-10^9, often 10^{10}-10^{11} cfu/ml), unrepresentative of natural levels (maximally 10^4-10^5 cfu/ml in rain water);

(c) artificial methods of inoculum delivery (e.g. stabbing stems or twigs or leaves with heavily inoculated needles or injecting bacterial suspensions with hypodermic needles), which bypass epidermal, bark or other structural barriers to infection.

At high inoculum concentration, pathogenicity can be easily confused with the hypersensitive reaction (HR), a common resistance response of plants to heterologous pathogens (i.e., strains which infect host(s) other than the one being tested). HR, which was not known as such prior to the mid-60's, occurs with most bacterial and fungal plant pathogens on their non-host plants and, when the infected inoculum concentration is $5x10^6$ to 10^7 cfu/ml or greater, is visible to the naked eye as localized necrosis and senescence of the inoculated tissue. Adding to the above confusion are several cases of misidentification. Bacteriological-nutritional tests that distinguish P. s. syringae from other closely related plant pathogenic bacteria currently classified as "pathovars" of the P. syringae group were inadequate or unavailable in the past. Although this situation has improved considerably in recent years, the key tests are not routinely employed by workers who are not expressly interested in taxonomy or host specificity.

In summary, some strains of P. s. syringae have a quite narrow host range and show a striking correlation between pathogenicity and host of origin, while other strains appear to have a broader host range, the limits of which are

uncertain in most cases. Disease caused by pathogenic P. s. syringae strains on many woody and herbaceous plants is rare in California and probably occurred only after predisposition by frost injury.

All strains used in the experiment will be isolated from the surfaces of healthy plants, not from any disease lesions. Dr. Lindow has screened a collection of such P. s. syringae isolates for pathogenicity towards oats, barley, corn, tomato, wheat, citrus, almond and three cultivars of beans. Most isolates were not pathogenic on any of these hosts while others showed different specificity patterns. None of the isolates was pathogenic on all hosts that were tested. Only strains shown not to be pathogenic to any of these crops will be used for the proposed field trials. The use of non-virulent non-toxin-producing strains (see paragraphs 4 and 5 below), in a location where there are no susceptible crops will protect crop plants surrounding the test site. (I) Among P. syringae strains known to cause plant disease, no correlation exists as to ice phenotype. INA^+ are just as likely to cause disease as are INA^-, especially those that cause diseases of cereal grains. Bacterial plant diseases are rare or absent in the Tulelake Basin because the dry, high desert climate is not conducive to development of bacterial diseases.

3. Epiphytic Biology

P. s. syringae colonizes the leaf surfaces of host and non-host plants in what has been called "resident phase." According to several reports, isolates from resident phase populations are usually specific, with respect to

pathogenicity, to that host. It should be noted that pathogenicity is not a prerequisite for epiphytic growth on herbaceous plants. It is possible that pathogenic capability gives some strains an advantage as epiphytes or that their epiphytic fitness is a significant component of their host range under natural conditions.

Due to their ice nucleating properties, epiphytic populations of INA^+ P. s. syringae predispose frost-sensitive plants to freezing injury at relatively warm subzero temperatures. This might be considered an "opportunistic" type of pathogenicity. (I)

4. Frost-conditional Virulence

Frost injury has often been reported as a predisposing factor for infection of some plants by certain P. s. syringae on several hosts (e.g. pear, apricot, peach). Ice formation is either required for or favors disease development in these cases. Most of those studies were conducted prior to the discovery of ice nucleation as a property of P. s. syringae or the availability of ice nucleation deficient mutants of the bacterium. Evidence, although circumstantial, is highly suggestive that ice nucleation activity is a frost-conditional virulence determinant of certain P. s. syringae under field conditions. Therefore, the INA^- deletion mutants to be tested, in addition to being unable to cause frost injury, are expected to be less likely than the INA^+ bacteria to cause disease. (I)

5. Phytotoxins

Strains of P. s. syringae produce low molecular weight peptide phytotoxins (syringomycin or syringotoxin, depending on their host of origin). Several reports suggest that these toxins are either required or accessory factors for pathogenicity on most, but apparently not on all hosts of P. s. syringae. Among over 100 isolates recently tested in the laboratory of Dr. Lindow, approximately 50 percent did not produce these toxins in culture. Many isolates are known to lose toxin production readily. Strains that do not produce toxins will be used in the proposed field tests. (I)

B. Erwinia herbicola

E. herbicola comprises yellow pigmented bacteria commonly epiphytic on plants. Although E. herbicola is commonly found in association with diseased tissue, it is mostly an opportunistic secondary organism. There are a few documented instances of plant pathogenicity of this bacterium, but none in California. In other cases it appears to effect a certain degree of biological protection against primary bacterial pathogens, such as Erwinia amylovora on pear. There is some uncertainty regarding the possible relatedness of yellow pigmented plant isolates classified as E. herbicola to similar isolates of clinical origin classified as Enterobacter agglomerans. There have been no reports of pathogenicity of plant isolates to man or animals or of human isolates to plants. Most isolates of E. herbicola of plant origin grow optimally at or near 28-30° C. and only sparsely, if at all, at 37° C. This supports the prevailing view that pathogenicity of yellow pigmented isolates classified as Erwinia herbicola or Enterobacter agglomerans to man or warm-blooded

animals appears to be a property of isolates of human or animal origin but not of plant origin. The strains to be used in this experiment were isolated from healthy plants. Due to its ice nucleating properties, E. herbicola contributes to frost damage to plants. (I)

5.2.2 Construction of INA Deletion Mutants

The purpose of the experiment is to evaluate the efficacy of INA^- deletion mutants of selected prototype strains of P. syringae pv. syringae and E. herbicola as biological competitors of epiphytic components of INA^+ populations occurring naturally. The construction of INA^- deletion mutants from INA^+ isolated bacteria does not add new or exotic properties which would make the mutant more competitive than its parent bacteria. The mutation only deletes functions from the treated bacteria. The advantages of such constructed mutants compared to chemically-induced equivalents are genetic stability and the absence of silent mutations which may adversely affect competitive fitness.

The strategy for constructing such mutants will involve:

(i) Construction of deletions covering all or part of the INA region carried on recombinant plasmid inserts.

(ii) Introduction of the above derived plasmids in the homologous strain or closely related strains of the same species or pathovar via conjugation or transformation. The regions of DNA homology flanking the deletion on the cloned insert are expected to promote reciprocal exchange of DNA between the plasmid and the chromosome so that the deletion will be transferred to the chromosome and the wild type region to the vector plasmid.

(iii) Elimination of the vector carrying the genomic INA^+ region by growth on media that does not select for its presence within a cell.

To facilitate population monitoring the researchers will select for the deletion bacterial strains carrying spontaneous resistance to certain antibiotics. This resistance will be unaffected by the deletion to be performed, and will enable the researchers to isolate selectively the bacteria released for confirmation of identity and phenotype by Southern blot hybridization analysis. This technique facilitates the large-scale monitoring of bacterial populations on a routine basis to determine survival and lateral dissemination of strains.

The DNA inserts will be transferred to bacterial plasmid vectors best suited for the construction. All plasmid and strain constructions described above will be done in the laboratories of Drs. Lindow and Panopoulos (not at the test site) and will be verified by molecular genetic tests and criteria including restriction endonuclease digestions, gel electrophoresis, and blot hybridizations. (I)

5.2.3 Previous Field Tests With INA^- Bacteria

A cruder form of the proposed experiment has already been field tested. In the earlier experiment, Drs. Lindow and Panopoulos used chemicals to mutate the naturally-occurring INA^+ Pseudomonas syringae and E. herbicola to INA^- bacteria. Ultraviolet irradiation and chemical mutagenesis are imprecise mutational procedures as they cause random mutations. Nevertheless, suitable INA^- mutants were isolated and field tested for their efficacy in preventing

frost damage to commercially significant crops. These mutant bacteria and wild-type bacteria have been sprayed on plants at the same test site in individual experiments over the past several years and no environmental harm has resulted. Bacteria were applied at the same times of year, with the same equipment, at the same concentration, and on the same plant varieties as proposed for the field test. Past studies with genetically marked strains have shown that the bacteria do not survive well through a full yearly cycle, i.e., their populations drop to very low levels during the winter season. These epiphytic bacteria do not survive for more than a month in the soil. Spray-applied INA^- bacteria could not be detected even immediately adjacent to the inoculated plot. No INA^- mutants could be detected in the area in subsequent years. The bacteria can survive up to a year in plant debris on the surface of the soil, but die rapidly (within a month) when plant debris is incorporated into the soil.

5.3 Effects of the Proposed Field Test

5.3.1 Risk Assessment

A. The Method

An analysis which appeared in Section VII of the October 1977, NIH Environmental Impact Statement on NIH Guidelines for Research Involving Recombinant DNA Molecules is paraphrased here. A series of events must occur in order for there to be harm from a recombinant DNA experiment; the overall probability of a detrimental effect will be the product of the probabilities of the individual steps. If the probability of any one of these essential steps

is zero, the product (the probability of harm) will be zero. If one or more of the factors is very improbable (probability close to zero), the probability of harm for the overall process will be extremely small as well.

The initial steps in this analysis will be common to all recombinant DNA experiments: a) The organism must contain foreign genes, that is, have some unique property as a result of recombinant DNA rearrangements, not present in naturally occurring organisms. b) The organism must escape from the experimental situation. c) The organism must survive after escape. d) The organism or the recombinant DNA specifying the harmful product must become established in an environment.

The general applicability of these four steps serve as the basis for the specific containment procedures recommended in the Guidelines for organisms containing recombinant DNA. Thus, laboratory experiments are designed to minimize factor (b) (escape from the experimental situation) by the use of appropriate physical containment techniques. The likelihood of steps (c) and (d) are reflected in the estimate of the biological containment of a particular system. If an organism escapes, but cannot survive or grow sufficiently to establish itself, or, alternatively, pass on the recombinant DNA to some other organism, it can have no long-term effect on man or the environment. Organisms containing recombinant DNA have a potential to affect the environment only if the organisms have the capacity to survive and replicate; if not, the probability of harm approaches zero. The concept embodied in (a), that organisms are subject to special oversight only if they are in some sense unique (contain foreign genes), is reflected in the exemption of many recombinant DNA experiments from the requirements of the Guidelines. For

example, "self-cloning," rearrangements within an organism of its own DNA, do not add new information, and are likely to have already arisen spontaneously in a population. Such experiments have been exempt from most of the requirements of the Guidelines since the 1978 revision (section III-D-3 in the current Guidelines). Similarly, experiments which involve moving DNA, by recombinant DNA techniques, from one organism to another with which it normally exchanges genetic information, is not likely to create a unique organism, and therefore has been exempted from the Guidelines (section III-D-4 in the current Guidelines).

If the probability of one or more of these initial four factors (a-d) is zero, the probability of harm from that particular recombinant becomes zero. If all four factors are significantly above zero, then the probability of the organism maintaining itself in the environment will be reasonably high, and a careful consideration of the environmental effects of that organism should be undertaken. An analysis of possible outcomes should consider the interactions of the recombinant organism with other organisms in the environment, including possible mechanisms for exchange of DNA, the expression of new and possibly harmful products by the organisms, and the potential for competitive growth of the organism.

B. Application of the Method to the Proposed Experiment

1. Presence of Foreign DNA

Because the bacteria in this proposed field test represent a single deletion of chromosomal information, no functional foreign gene has been added.

Mutations in the genes for ice nucleation have been made previously through techniques other than recombinant DNA, and naturally occurring mutations, including deletions, happen at low but measurable frequencies in nature. Indeed, INA$^-$ strains of the bacteria to be used are found in all environments and can represent up to 80 percent of all P. syringae pv. syringae at a given location. Therefore, the recombinant DNA constructed deletion derivative can not be considered unique. Under the current Guidelines, the construction of such bacteria in the laboratory is considered a "self-cloning" experiment, and therefore is totally exempt from the Guidelines under section III-D-3. Any experiment, however, which proposes to take such an organism out of the laboratory, is no longer exempt from the Guidelines, but rather must undergo RAC and NIH review. Therefore, this experiment is exempt for laboratory work, but requires RAC and NIH review for field testing. That review, however, has recognized that the organism to be tested does not contain a foreign gene and is not unique. Therefore, factor (a) in the risk assessment method described in Section 5.3.1A above, should be considered to be zero.

2. Escape from the Experimental Site

The probability that at least a few organisms will escape from the test plot is high. The probability that significant numbers of the INA$^-$ bacteria will escape, however, is low. The test plot and the possible effects in the event of escape are described below.

3. Survival of Organisms After Escape, and Establishment in the Environment

Past experiments and observations show that the organisms may survive for a while, although probably not through a full yearly cycle and in low numbers. Because the deletion is carried in chromosomal DNA, there is not much likelihood for transfer of the recombinant information to other organisms. In order to become established, the deletion mutants would have to compete successfully against much larger numbers of the naturally occurring epiphytic bacteria including INA^+ and INA^- phenotypes of the same species and other wild bacterial species. The INA deletion mutants are unlikely to become established because of the preexisting colonization of available plant surfaces by these naturally-occurring epiphytic bacteria.

C. Probable Risk

The probability of any adverse environmental effect of this field test is at or near zero based on assessment of risk using the probability method described above. Although the probable risk is low, this Environmental Assessment includes the following detailed discussion of how the proposed experiment will affect the environment in which it will be conducted.

5.3.2 The Test Plot

The field tests will be conducted on small plots within a 100-acre tract. The location of this tract is described in Section 5.1. Identical plots will be established for potatoes, snapbeans and tomatoes. Each plot will contain nine different treatments which will be applied in a randomized complete block design with four replications (see plot diagram and description of treatments attached as Appendix C). Of the nine "treatments" used for each plot, numbers

2-5 will involve the spraying of the modified INA^- bacteria. Other "treatments" include untreated controls (number 1), plants treated only with INA^+ bacteria (number 6), spraying with INA^- bacteria mutagenized with chemicals (number 7), spraying with a naturally occurring bacterium (P. fluorescens) (number 8), and spraying with bactericides (number 9). Each replication of each treatment will include four 40-foot rows with the plants spaced approximately one foot apart. Two rows of plants for each treatment would be established in early May and two rows would be established in mid July, except tomato plants would only be planted in July to be subject to the fall frost. Since each treatment is replicated four times within the plot, the total area of any one treatment is 0.044 acres and the size of the total planted area for each plot is 0.04 acres. In addition to the sixteen rows of treated and controlled plants on each plot, an additional three rows of plants will be used on each side of the plot and five plants on the end of each row to guard against wind, dust and other intrusions into the plot. Twenty feet of cultivated barren soil will surround each plot and this barren area will be surrounded by a ten-foot wide dirt road.

The INA^- deletion mutants will be sprayed on the leaves of the emerging plants or applied to seed pieces when planted. If the experiment is successful, the INA^- bacteria will colonize the plants to the exclusion of colonization by the INA^+ bacteria and protect the plants from frost damage. Whether or not this occurs, the INA^- bacteria will not harm the plants, because the bacteria are not pathogenic to potato, tomato, or beans, the plants that will be used in the field test.

Some of the modified bacteria may be carried to other parts of the test plot by insects, birds, and the wind. This will have no effect on the plot,

because nothing else is planted there and, in any event, the strain to be used has been screened for pathogenicity to all major crops in the area and shown to be nonpathogenetic to those crops. Furthermore, the modified bacteria are specifically adapted to the target plants and exist in such small numbers compared to naturally occurring bacteria that they could not effectively colonize either crops or non-commercial plant species in the area. Previous studies have shown that epiphytic bacteria do not survive for more than a month in the soil. The bacteria can survive up to a year in plant debris on the surface of the soil, but die rapidly when plant debris is incorporated into the soil.

5.3.3 Crops and Non-Commercial Plant Species

Some strains or isolates of P. s. syringae have been reported to be pathogenic on oats, wheat and barley, crops that are grown in the agricultural areas surrounding the Tulelake Research Station. The strains to be used in the experiment will have been screened for pathogenicity towards oats, wheat, barley, corn, tomato and three cultivars of beans. It is possible that small numbers of the INA^- bacteria will be carried away from the test plot to surrounding crops or non-commercial plant species by wind, birds and insects. The numbers of bacteria involved would be very small in comparison to the wild-type bacteria that already exist in nature (P. syringae, for example is ubiquitous). It is highly unlikely that all of the deletion mutants that leave the test plot would be deposited in one place. Even if this did occur, there would be a negligible chance that there would be sufficient numbers of the deletion mutants to significantly affect the ratio between INA^+ and INA^- wild-type bacteria. In addition, it is highly unlikely that the modified bacteria

could successfully colonize any plant other than potatoes, tomatoes, or beans since the bacterial strain is adapted best to the target plant and other plants are already colonized by other bacteria. If there was colonization by the deletion mutants the result would be either conditional protection of the crops from frost damage or no effect. The foregoing discussion applies equally to non-commercial plants. Moreover, no known bacterial diseases occur in conifers, which dominate the forest around Tulelake Basin.

Erwinia herbicola is not pathogenic to plants in Northern California. Therefore, no plant diseases are expected from application of E. herbicola in the proposed field test. Moreover, the same factors limiting colonization or spread of P. s. syringae INA^- mutants would limit the spread of E. herbicola INA^- mutants.

5.3.4 Local Epiphytic Bacterial Populations

P. s. syringae and Erwinia herbicola occur naturally in the vicinity of the test site. Under California conditions, a large seasonal variation in the numbers of epiphytic ice-nucleation-active bacteria on both annual and perennial plants has been observed. The bacterial populations found on healthy pear flowers and leaves are typical of this variation. Populations of ice-nucleation-active-bacteria are generally low (less than 100 cells/g fresh weight of leaf or bud tissue) on overwintering plant tissues of deciduous plants or on emerging cotyledons or leaves of annual plants. However, large epiphytic populations of such bacteria (principally P. syringae) are present on emerging flowers and/or leaves of these plants. A 100-fold increase in bacterial populations has occurred on pear during the 2-week period following

bud break. Populations of ice-nucleation-active-bacteria have been shown to be largest during April through May, coinciding with the period of maximum frost hazard to pear in this location. Populations of ice-nucleation-active bacteria decreased after late May, declining with the onset of hot, dry weather to 100 cells/g by late summer.

It should be noted that not all INA^+ bacterial cells are active as ice nuclei foci while on plant surfaces. Nucleation frequency is not an intrinsic, invariable property of the bacteria, but a property that varies, depending on the temperature and other factors. In an experiment by Drs. Lindow and Panopoulos with pear, only about one P. syringae cell in 1000 acted as an ice nucleus at -5° C while on plant surfaces. The frost sensitivity of most plants can be explained by the fact they harbor very large numbers of ice-nucleation-active bacteria. Ice nucleation strains of both P. s. syringae and E. herbicola can be detected on most plants, but P. s. syringae is the predominant species on the majority of plant species investigated in California. (0)

Observations of naturally-occurring bacterial populations on potato plants near Tulelake in Northern California are consistent with the pear studies. INA^+ populations were very low (30 cells/gram fresh weight) shortly after emergence of the plants, but increased rapidly during periods of cool wet weather. INA^+ bacteria nevertheless generally comprised less than 0.01 percent of the total bacteria on leaves of potato plants. During the 1979 growing season, strains of P. syringae, E. herbicola, and P. fluorescens were found in nearly equal numbers on potato leaves. The number of ice nuclei active at -5° C was substantially less than 1.0 percent of the number of INA^+ bacteria during

most of the growing season. This implies an ice nucleation frequency of approximately one ice nucleus per 100 INA^+ bacteria cells on potato in the field and resembles the nucleation frequency for a strain of P. s. syringae at -5° C measured under greenhouse conditions. Measured nucleation frequency of a strain of E. herbicola at -5° C under greenhouse conditions, in contrast, was only one active ice nucleus per 1000 cells approximately. (W)

If the field test is successful, the bacteria that have had their ice-nucleation genes removed will colonize plants first and prevent the naturally-occurring INA^+ from colonizing and becoming established on the plants, thereby protecting them from frost damage. The modified bacteria will be no more competitive, and probably less so, than their naturally-occurring INA^+ counterparts because they are adapted only to the target crop for the experiment. It is believed that INA^+ bacteria are more competitive than INA^- bacteria because of their ability to induce frost damage and obtain nutrients through lesions caused by the frost damage. Because of this comparative lack of competitiveness and the small numbers of the modified bacteria in relation to the naturally occurring, ubiquitous bacteria, there is no significant risk that the modified bacteria will have an adverse effect upon existing epiphytic populations.

5.3.5 Insect Populations

Mechanisms of frost hardiness in insects closely parallel those in plants (Asahina, 1966; Salt, 1961). Frost injury can be avoided in those insects that tolerate ice formation within their tissues or that escape all ice formation by supercooling (Asahina, 1966; Salt, 1961, 1962; Salt and Kumic, 1961;

Zachariassen and Hammel, 1976). Many insects can supercool to very low temperatures (less than -20° C) (Salt, 1961). However, since most of these species must supercool to survive, factors that decrease the supercooling capacity of these insects would reduce their survival. Feeding has been shown to decrease supercooling capacity (Salt, 1953, 1958, 1961, 1966a, b; 1968). Ice nucleation usually has been associated with undigested food in the gut. In fact, the supercooling point of insects was found to decrease with an increasing number of nucleating particles in the insects (Salt, 1979). There is circumstantial evidence that leaf-surface ice-nucleation-active bacteria may play at least a partial role in the limitation of supercooling of leaf-feeding insects. Analogous to plant frost survival, the presence of INA^+ bacteria would be detrimental to survival of frost-sensitive insects and possibly beneficial to frost-tolerant insect species. (T)

To the extent the field test is successful and the modified INA^- bacteria prevent INA^+ bacteria from growing on treated plants, the opposite effect may occur; that is, frost sensitive insects that feed on the leaves that are sprayed might have a better chance of survival. Given the small numbers of the modified bacteria expected to leave the test plot in relation to naturally occurring INA^+ and INA^- bacteria, however, the modified bacteria would not change whatever balance exists between INA^+ and INA^- bacteria in the area outside the test plot. In any event, insect populations in the area are systematically controlled by application of pesticides to the more than 90 percent of land in Tulelake Basin under agricultural production. (X) Thus, any effect that the INA^- bacteria would have on insects in the area would be insignificant, being limited to the relatively small number of insects that would feed on the small number of plants sprayed with the modified INA^-

bacteria. That small number of insects would not change the overall balance in the area between frost-sensitive and frost-tolerant insects. Fields treated conventionally in agriculture with bactericides, pruned, or other agronomic practices that reduce INA^+ bacterial populations would be expected to affect insect populations if there was any causal relationship. No such observations have been reported in the world literature.

5.3.6 Animal Populations

The modified INA^- bacteria to be used in the experiment will not differ from their counterparts that already exist in nature. There are no known cases of either P. s. syringae or Erwinia herbicola, isolated from plants, having any effect on animals. Wild-type strains of both bacteria have been sprayed on test plots by Drs. Lindow and Panopoulos over several years and by others over a period of 20 years with no adverse effects on animals or humans. P. s. syringae does not survive at mammalian body temperatures and has no known pathogenicity to cold-blooded animals. E. herbicola isolated from plants, as is the case for the proposed field test, have no known pathogenicity to animals. (U) Accordingly, the proposed experiment will have no significant effect on animals or humans.

5.3.7 Atmosphere and Climate

Recent work has shown that significant numbers of leaf-surface bacteria, including species of ice-nucleation-active bacteria, can enter the atmosphere as aerosols (Lindemann et al., 1979, 1981; Vennette and Kennedy, 1975). The settling rate of these small aerosolized bacteria in the atmosphere was shown

to be slow, allowing considerable horizontal movement in even modest winds (Vennette and Kennedy, 1975). Rainsplash has been considered a primary mechanism of generating aerosols containing bacteria, but more recent research indicates that high numbers of ice nucleation-active strains of P. s. syringae can enter the atmosphere from dry plant surfaces (Lindemann et al. 1979, 1981) as well as from rainsplash (Sands, 1981). (T)

Long distance transport of ice-nucleation-active bacteria through the atmosphere appears possible. Ice-nucleation-active bacteria have an extensive worldwide distribution on plants and these bacteria have been detected in the atmosphere in diverse parts of the globe removed from plant sources of these bacteria (Flanagen and Jayaweera, 1980; Savena, 1981). Indeed, the ubiquity of these species may be due in part to such long distance transport. Many bacteria have been shown to be sensitive to desiccation and ultraviolet light while suspended in aerosols (Gregory, 1973) and have only a limited life expectancy while in the air. Should this also be the case for ice-nucleation-active bacterial species, it would be advantageous if they could be removed from the atmosphere and deposited on plant surfaces that probably provide a more hospitable environment for growth and survival. As small aerosol particles, bacteria would not sediment rapidly from the upper atmosphere, but could be removed rapidly in precipitation. (T)

The presence of ice nuclei in the atmosphere is important in precipitation processes. Much water in the atmosphere exists as small droplets of supercooled water or as water vapor in equilibrium with this supercooled water (Bigg, 1953). Significant deposition of these water droplets occurs only when they increase in size, increasing their rate of settling (Hobbs, 1974). One

major mechanism for the process requires ice nuclei to initiate ice formation in some of these droplets which then increase in size by collision with other droplets of supercooled water or by deposition of water vapor on the particles that have a lower vapor pressure than supercooled water at the temperatures from -5° to -20° C in clouds. The most abundant and only detectable ice nuclei found in the atmosphere to date are inorganic particles. (T)

Much circumstantial evidence indicates ice-nucleation-active bacteria may be important in atmospheric precipitation processes. Several researchers have suggested that ice nucleation-active bacteria may be important in atmospheric precipitation processes because they contribute ice nuclei active at warm temperatures (Lindow et al., 1975b; Maki et al., 1974; Schnell et al., 1981; Vali and Schnell, 1975; Zankofsky, et al., 1981). P. s. syringae have been shown to be active as ice nuclei as aerosols in cloud chamber tests that are similar to conditions found in the atmosphere (Maki and Willoughby, 1978; Schnell, 1976; Schnell et al., 1981). Thus, since ice-nucleation-active bacteria have been reported in significant numbers in the atmosphere, there is good circumstantial evidence that plant sources of ice-nucleation-active bacteria may be important as atmospheric ice nucleants and thus as a factor in determining global climatology by affecting worldwide precipitation processes. (T)

It is conceivable that P. s. pv. syringae and other ice-nucleation-active bacteria would derive a survival benefit from nucleating ice formation in the atmosphere. Assuming that ice-nucleation-active bacteria would benefit by distribution on leaf surfaces by long-distance transport in the upper atmosphere, factors increasing the survival of these bacteria during this

process would be beneficial. Ice nucleation-active bacteria would be removed efficiently from the atmosphere by nucleating and being incorporated into supercooled water droplets, thus facilitating their removal from the upper atmosphere before viability was lost in the harsh environment of the atmosphere. Therefore, ice-nucleation activity could have evolved as a factor increasing the efficiency of distribution of these bacteria via atmospheric transportation processes. In addition, by increasing precipitation or altering precipitation patterns, these bacteria may have improved plant growth or environmental conditions on plant surfaces to allow better survival or growth of ice-nucleation-active bacteria on plants (Sands 1981). (T)

The modified INA^- bacteria are subject to dispersal into the atmosphere in the same manner as wild INA^- and INA^+ bacteria. The modified INA^- bacteria to be released in this field test will be no more competitive than their wild counterparts and, because of their limited numbers, will not have an effect on whatever balance exists between INA^+ and INA^- bacteria on plants in nature. There is no evidence that INA^- bacteria displace INA^+ bacteria in the atmosphere. Only in the unlikely event that INA^- bacteria were to displace INA^+ bacteria over large areas of the world's vegetation could any impact on INA^+ populations in the atmosphere occur. Routine agricultural practices such as cultivation, pruning, application of pesticides and herbicides, land clearing and burning remove many times more INA^+ bacteria from vegetation than the numbers that might be prevented from colonizing plants by these proposed field tests. No change in weather or climate has been attributed to these other man-made changes in INA^+ bacterial populations. Since the survival time for bacteria in the atmosphere is limited and INA^+ bacteria have much greater ice nucleating ability than INA^- bacteria to form precipitation droplets to

carry the bacteria back to earth, INA^- bacteria, including the modified INA^- bacteria will be at a competitive disadvantage in the atmosphere.

5.3.8 Scientific and Agricultural Benefits

If the experiment is successful, it may lead to a cost-effective, energy-saving method of preventing frost damage to plants. Plant frost injury at temperatures above -5° C (23° F) in the United States varies from year to year, but estimates of average annual losses range from one to three billion dollars. Worldwide agricultural losses due to frost injury are much higher. In addition to direct losses due to frost injury, hundreds of millions of dollars are spent yearly in attempts to reduce frost injury. Methods such as the use of wind machines to mix warm air aloft with cold air near the ground, burning of fossil fuels to directly heat the air near crop plants, and pumping large amounts of water to reduce the latent heat of fusion on or near plants are not especially effective, are costly, and potentially can reduce environmental quality. (0)

Replacement of these methods with a more effective, less costly method of frost prevention would significantly lessen agricultural losses to frost damage, result in lower food prices and ensure greater food supplies for the world's increasing population. The success or failure of the field test cannot be predicted in advance with certainty. However, prior experiments in the laboratory and field tests with INA^- mutants achieved through chemical means, indicate a good chance of success. Whether or not the proposed field test is successful, it will add to knowledge of the interaction of INA^+ and INA^- bacteria and plants. This may contribute ultimately to a solution to the frost

damage problem through the use of bacteria modified by recombinant DNA techniques or it may lead to frost damage experiments involving different frost prevention methods.

If the field test is not conducted, it will not be known whether it is possible to develop this technology that promises to prevent frost damage without unreasonable economic or environmental costs.

5.3.9 Worst Case Considerations

No worst case analysis is required, because NIH is not considering significant adverse effects on the human environment in connection with an environmental impact statement and there is no substantial uncertainty about either the likelihood or magnitude of environmental harm that might occur from the field test. Nevertheless, the following observations are presented, based on the analysis in this assessment.

As noted in Section 5.3.3, it is remotely possible, although highly unlikely, that the modified bacteria could be transported off site to a neighboring field in sufficient numbers to become established. Not only is it unlikely that a significant number would be transported from the site, it is unlikely that, if they were, they would be deposited in the same location in sufficient numbers to become established, particularly since plant surfaces will already be occupied by much larger populations of naturally-occurring epiphytic bacteria.

If, however, the modified bacteria were to escape the monitoring network and were to become established in neighboring fields, it is conceivable that the modified bacteria, together with INA^- bacteria of the same or different species could alter whatever balance may exist in the particular field between INA^+ and INA^- bacteria. The scientific literature, however, does not indicate that there is any stable balance in the environment or in a field between INA^+ and INA^- bacteria. Large seasonal fluctuations and widely varying proportions of INA^+ and INA^- bacteria in a given field appear to be characteristic in nature, as bacterial populations change in response to environmental variables such as temperature and humidity. Among P. s. syringae, the INA^+ phenotype may be dominant in one field at a given time, while the INA^- phenotype is dominant in other fields in the vicinity, and the pattern may change over time with no perceptible environmental consequences, other than greater frost injury in INA^+ fields. Existing agricultural practices add a further variable that drastically affects natural bacterial populations without any known adverse environmental consequences.

Even if there were some fixed INA^+/INA^- balance in nature that had some environmental significance, it is unlikely that the slight alteration of that balance that could conceivably result if modified bacteria from this field test become established in adjacent fields would have any environmental consequences. The numbers to be applied are many orders of magnitude lower than the number of naturally-occurring bacteria, both INA^+ and INA^-, against which they would have to compete for space and nutrients for survival, growth and reproduction. Nothing in this proposed field test will enhance the reproductive potential or competitive fitness of the modified INA^- bacteria. In all respects, it will be identical to its wild counterparts, except that it

will be unable to ice nucleate. It will therefore be subject to the same environmental factors and the same population control mechanisms as the much more numerous wild bacteria. To the extent that the INA^+ characteristic plays a role in growth, survival or reproduction, the modified bacteria will be more vulnerable to environmental and other stresses than its wild counterparts. Therefore, any escape of modified bacteria from the test site would not cause any significant environmental impacts.

5.4 Effects of Alternatives

5.4.1 Approval of Original Proposal Without Restriction

As originally proposed in the application dated September 17, 1982, the field test would have been basically the same as the field test approved by NIH on June 1, 1983, except that it would be conducted at six sites instead of one, and antibiotics would be sprayed on the plants to kill the bacteria in the unlikely event the bacteria caused some harm to the plants.

Conducting field tests at six locations instead of one would increase the number of modified bacteria being tested and thus increase the probability that the INA^- bacteria might escape from one of the test sites. In addition, the logistics of monitoring experiments at six locations would be more difficult.

The RAC recommended that in the unlikely event that an "emergency plan" is necessary, antibiotics should not be used, and that burning or burying were preferred.

5.4.2 Approval of Modified Proposal Without Restriction

Under this alternative, the field test would be the same as that approved by NIH on June 1, 1983, except that antibiotics would be sprayed on the plants to kill the bacteria in the unlikely event that the plants were harmed. In addition, plants outside the area of application from which the modified bacteria can be isolated would be treated with the antibiotics if it is necessary to quickly terminate the experiment. The use of antibiotics was judged to be an environmentally invasive procedure compared to burning or burying.

5.4.3 Approval of Proposal as Approved by NIH on June 1, 1983, With Changes Proposed by the Investigators

Under this alternative the full test as approved by NIH on June 1, 1983, would be approved with the restrictions proposed by the researchers that are described in Section 4.2.3 above.

Elimination of the "flagged" deletion monitoring method for at least the first twelve-month period of the field test will not pose any significant risk to the environment or increase the potential for such a risk. "Flagged" deletions would have been advantageous only in the event that large numbers of different deletion mutants would be used, by making possible unique Southern blot hybridization methods and probes to differentiate the various INA^- strains. Since only two recombinant DNA constructed INA^- deletion strains are anticipated to be released at this time, no compelling need exists for the

procedures. Use of an antibiotic resistance marker will permit prompt and accurate identification of the two INA$^-$ strains.

Eliminating the field testing of Erwinia herbicola for at least the first year of the field test will simplify monitoring efforts because it will reduce the number and type of bacteria involved.

5.4.4 Withhold Approval

Under this alternative, only classical methods of frost control would be available. Since these methods are only partially effective and many use large amounts of energy, economic losses resulting from frost damage would continue and energy resources (water, fossil fuels) would continue to be expended. In addition, many of these classical methods, such as burning to heat air near the crop, can directly harm the environment.

Another effect of this alternative would be to retard scientific progress.

5.5 Summary and Conclusions

The INA$^-$ bacteria proposed to be used in the field test would be derived by the removal of the genetic material encoding the property (INA$^+$). No functional foreign genes would be added. Thus, no new phenotype would be introduced into the mutants used in the field test.

Large INA$^-$ populations of both P. s. syringae and E. herbicola already occur naturally. Depending on the host plant from which the bacteria are

isolated, such bacteria can represent, for example, up to 80 percent for P. s. pv. syringae and up to 100 percent for E. herbicola of the total number of epiphytes present. Such large ratios of INA^- bacteria in the environment suggests that some equilibrium already exists between INA^+ and INA^- strains and other bacteria. The populations proposed for field tests would be trivial introductions into the environment, and would not significantly affect that balance. Even if an imbalance in favor of the INA^- bacteria did occur on a local field, a situation that already exists in nature, the result would be protection of the plants from frost damage or no effect. Wild-type strains of these bacteria have been sprayed experimentally on plants for over 20 years in different biological control experiments in field tests. In most cases, it is not known whether or not these were INA^- or INA^-; the probability is that some of both have been used. No adverse effects on applicators, plants, animals or the environment have ever been reported. P. syringae pv. syringae has never been shown to be a pathogen of humans or animals and does not grow at mammalian body temperatures. E. herbicola strains of plant origin have never been shown to be associated with pathogenicity to humans or animals and the bacteria to be used in this experiment have been isolated from plants. (U) Some strains of P. syringae pv. syringae can cause disease in certain plants some of which are grown in the area surrounding the test plot, but the strains to be used in the proposed field test have been isolated from healthy plants and screened to assure that they do not cause disease to major crops grown in the area. Furthermore, there is some evidence that the bacteria are host specific, best suited for colonizing or causing disease only in the plant from which the bacteria were isolated. The modified INA^- bacteria are no more capable of causing plant disease than are their wild counterparts and will not change the balance between INA^+ and INA^- bacteria outside of the test plot. Since INA^-

strains of P. s. pv. syringae and E. herbicola already exist in nature, adding a few additional organisms in a field trial is highly unlikely to affect any precipitation patterns. The bacteria do not survive for long in the atmosphere and INA^- bacteria have a limited ability to initiate precipitation through ice nucleation and be carried back to earth by the precipitation.

Having completed the preceding environmental assessment, NIH finds again that approval of the modified proposal for a field test will not have a significant impact on living creatures (insects, animals, birds, man), plants, microorganisms or any other aspect of the environment and makes the same finding with respect to the alternative described in Sections 4.2.3.

6.0 Consultation

Those consulted in the preparation of this environmental assessment were:

Dr. Bernard Talbot, NIH
Dr. William Gartland, NIH
Dr. Elizabeth Milewski, NIH
Mr. Michael Kremer, NIH
Dr. Susan Gottesman, NIH
Dr. Sue Tolin, Department of Agriculture
Mr. Robert Lanman, Office of the General Counsel,
Department of Health and Human Services
Ms. Judith Bartnoff, Office of the United States
Attorney for the District of Columbia,
Department of Justice

Ms. J. Carol Williams, Land and Natural Resources Division, Department of Justice

Mr. William A. Anderson, II, Bracewell & Patterson

Dr. Steven E. Lindow, University of California, Berkeley

Dr. Nickolas J. Panopoulos, University of California, Berkeley

Dr. Anne Vidaver, Professor of Plant Pathology, University of Nebraska

Dr. Robert Haselkorn, F.L. Pritzker Distinguished Service Professor, Department of Molecular Genetics and Cell Biology, University of Chicago

Dr. Arthur Kelman, WARF Senior Research Professor, Department of Plant Pathology, University of Wisconsin-Madison

Dr. Albert H. Ellingboe, Professor of Plant Pathology, University of Wisconsin-Madison

Dr. Christen D. Upper, Professor of Plant Pathology, University of Wisconsin-Madison

Dr. David L. Coplin, Asscciate Professor, Department of Plant Pathology, Ohio State University, Ohio Agricultural Research and Development Center

7.0 References

All of the references tabulated below are available for public inspection at the NIH Office of Recombinant DNA Activities, Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland, 20205, on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

A Federal Register, Vol. 48, No. 106, Pp. 24548-24553, June 1, 1983.

B National Environmental Policy Act of 1969, as amended by Pub. L. 91-190, January 1, 1970; Pub. L. 94-52, July 3, 1975, and Pub. L. 94-83, August 9, 1975, 42 U.S.C. 4321-4327.

C Council on Environmental Quality Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act, 43 FR 55978-56007, November 29, 1978, 40 CFR Parts 1500-1508.

D Part 30, Environmental Protection, of the General Administration Manual of the Department of Health and Human Services, Transmittal No. 80.02 (5/1/80).

E September 17, 1982 submission from Drs. Steven Lindow and Nickolas Panopoulos to Director, Office of Recombinant DNA Activities, NIH

F Federal Register, Vol. 47, No. 1984, Pp. 41924-41925, September 22, 1982.

G Minutes of October 25, 1982, meeting of NIH Recombinant DNA Advisory Committee.

H Federal Register, Vol. 48, No. 6, Pp. 1158, January 10, 1983.

I March 8, 1983 submission from Drs. Steven Lindow and Nickolas Panopoulos to Director, Office of Recombinant DNA Activities, NIH.

J Federal Register, Vol. 48, No. 44, Pp. 9436-9441, March 4, 1983.

K Minutes of April 11, 1983, meeting of NIH Recombinant DNA Advisory Committee.

L April 6, 1983 letter from David L. Coplin to Anne Holiday Schauer.

M Foundation on Economic Trends, Jeremy Rifkin, Michael W. Fox, Environmental Action, Inc., Environmental Task Force, Plaintiffs, v. Margaret Heckler, James E. Wyngaarden, Richard Krause, Defendants, Civil Action No. 83-2714, United States District Court for the District of Columbia.

N Memorandum and Order, May 16, 1984, Civil Action No. 83-2714, United States District Court for the District of Columbia.

O Steven E. Lindow, "Methods of Preventing Frost Injury Caused by Epiphytic Ice-Nucleation-Active Bacteria," Plant Disease, March 1983, pp. 327-333.

P April 18, 1984, Declaration of Nina Fedoroff, Civil Action No. 83-2714, United States District Court for the District of Columbia.

Q Maps, Tulelake Quadrangle, Tule Lake Calif.-Oreg., N4145-W12115/15, U.S. Geological Survey (1951).

R Publication No. RF 11660-2, U.S. Fish and Wildlife Service, Department of the Interior (June 1983).

S Final Environmental Statement, Medicine Lake Planning Unit, Forest Service, U.S. Department of Agriculture (1978).

T Phytopathogenic Prokaryotes Vol 1, Chapter 14, Steven E. Lindow, "Epiphytic Ice Nucleation-Active Bacteria," Academic Press, 1982.

U April 19, 1984, Declaration of Anne Vidaver, filed in Civil Action No. 83-2714, United States District Court for the District of Columbia.

V Elizabeth Milewski and Bernard Talbot, "Proposals Involving Field Testing of Recombinant DNA Containing Organisms," Recombinant DNA Technical Bulletin, Vol. 6, No. 4, December, 1983 (NIH Publication No. 84-99).

W S.E. Lindow, "Population Dynamics of Epiphytic Ice Nuceation Active Bacteria on Frost Sensitive Plants and Frost Control by Means of Antagonistic Bacteria," in Plant Cold Hardiness and Freezing Stress, Academic Press, 1982.

X O.G. Bacon, et al., "Insects Affecting Potato: Biology and Control of the Green Peach Aphid," University of California, Davis, Department of Entomology.

Y September 21, 1984 letter from Steven Lindow to William Gartland.

Z C.S. Orser, et al., "Structural and Functional Analysis of the Pseudomonas syringae pv. syringae Ice Region and Construction of Ice Minus Deletion Mutants," Phytopathology, 74, 798, 1984.

Appendix A

Background Concerning the NIH Guidelines for Research Involving Recombinant DNA Molecules

The NIH Guidelines for Research Involving Recombinant DNA Molecules (Guidelines) were first issued on June 23, 1976, and were published in the Federal Register on July 7, 1976. The Guidelines were the product of a process of scientific and public discussions initiated in 1973 by scientists with concerns about the safety of the new recombinant DNA techniques. As stated in the "Decision of the Director, National Institutes of Health To Release Guidelines for Research on Recombinant DNA Molecules":

> "The object of these guidelines is to ensure that experimental DNA recombination will have no ill effects on those engaged in the work, on the general public, or on the environment . . . . It should be noted that the development of the guidelines was in large part tantamount to conducting an environmental impact assessment. For example, the objectives of recombinant DNA research and alternate approaches to reach those objectives, have been considered. The potential hazards and risks have been analyzed. Alternative approaches have been thoroughly considered to maximize safety and minimize potential risk. And an elaborate review structure has been created to achieve these safety objectives."

NIH published a Draft Environmental Impact Statement on the Guidelines in the Federal Register for public comment on September 9, 1976. After analysis

of the comments received a final Environmental Impact Statement (EIS) on NIH Guidelines for Research Involving Recombinant DNA Molecules was published in October, 1977.

On July 28, 1978, NIH published for public comment in the Federal Register (totalling 136 pages) proposed revised Guidelines together with "Decision of the Director, NIH, to Issue Revised Guidelines for Recombinant DNA Research," and an Environmental Impact Assessment. Following analysis of the public comments received, NIH published in the Federal Register on December 22, 1978, revised Guidelines together with "Decision of Director, NIH, to Issue Revised Guidelines for Recombinant DNA Research," and an Environmental Impact Assessment. The Guidelines have been revised many times since December, 1978. Copies of all revisions are available from the NIH Office of Recombinant DNA Activities, Building 31, Room 3B10, NIH, Bethesda, Maryland 20205. The most recent complete republication of the Guidelines was in the Federal Register of November 23, 1984.

The NIH Recombinant DNA Advisory Committee (RAC) consists of 25 voting members, appointed by the Secretary of the Department of Health and Human Services, including laymen and scientists of many different disciplines. The RAC also has non-voting liaison representatives from the following other Federal agencies:

Department of Agriculture
Department of Commerce
Department of Defense

Department of Health and Human Services

--Office of the Assistant Secretary for Health

--National Institutes of Health

--Centers for Disease Control

--National Institute for Occupational Safety and Health

--Food and Drug Administration

--National Center for Drugs and Biologics

Department of Energy

Department of Interior

Department of Justice

Department of Labor

Department of State

Department of Transportation

Environmental Protection Agency

Executive Office of the President

--Office of Science and Technology Policy

--Council on Environmental Quality

National Aeronautics and Space Administration

National Science Foundation

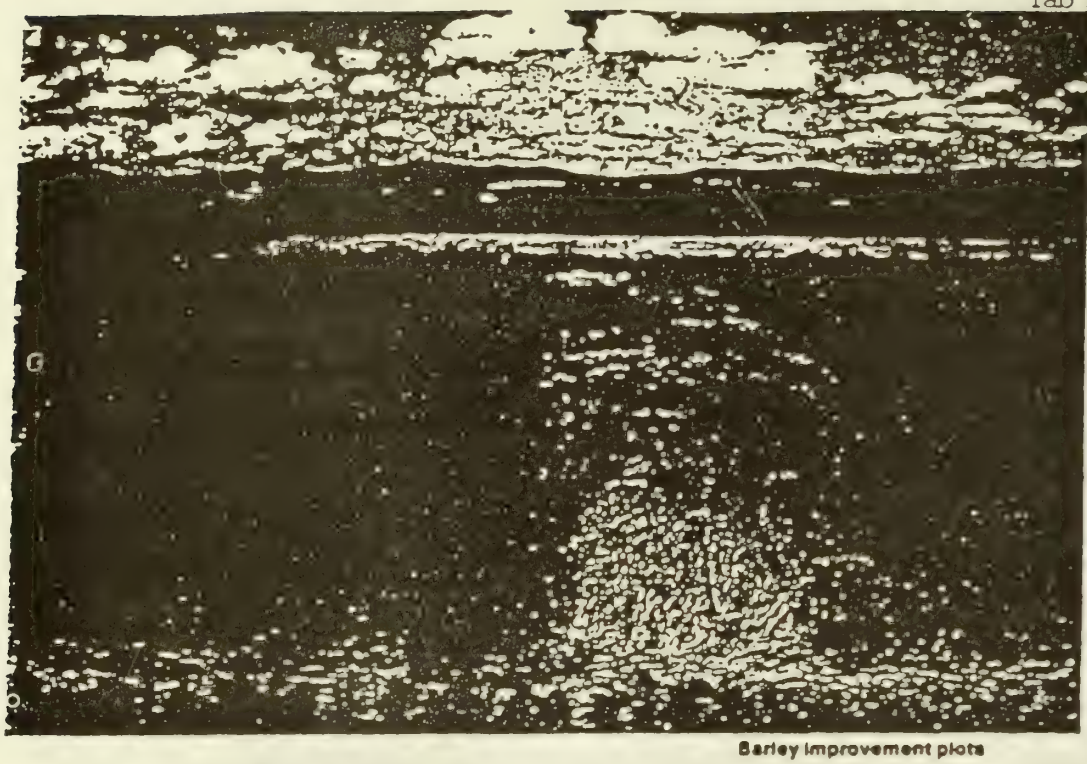
Nuclear Regulatory Commission

U.S. Arms Control and Disarmament Agency

Veterans Administration

A list of all current and past RAC members is available from ORDA at the address given above.

The NIH Guidelines for Research Involving Recombinant DNA Research are primarily safety standards for laboratory research, designed to keep organisms containing recombinant DNA within the laboratory. There is a section of the Guidelines, however, which deals with "deliberate release into the environment of any organisms containing recombinant DNA." (This was Section III-A, "Experiments that are not to be performed" in the original Guidelines of July 7, 1976; it was Section I-D-4 in the Guidelines of December 22, 1978; and it is Section III-A-2 in the Guidelines of November 23, 1984. The procedures for approval of "deliberate release into the environment" of any microorganism containing recombinant DNA, have remained the same since the December 1978 revision of the Guidelines; they are that "experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of the proposal in the Federal Register for 30 days of comment, review by the RAC, and a specific approval by NIH." The standard for approval of such "deliberate release into the environment of any organisms containing recombinant DNA" has remained the same since the December 1978 revision of the Guidelines; it is that the NIH "Director or a designee shall weigh each proposed action through appropriate analysis and consultation, to determine that it complies with the Guidelines and presents no significant risk to health or the environment."



Barley improvement plots

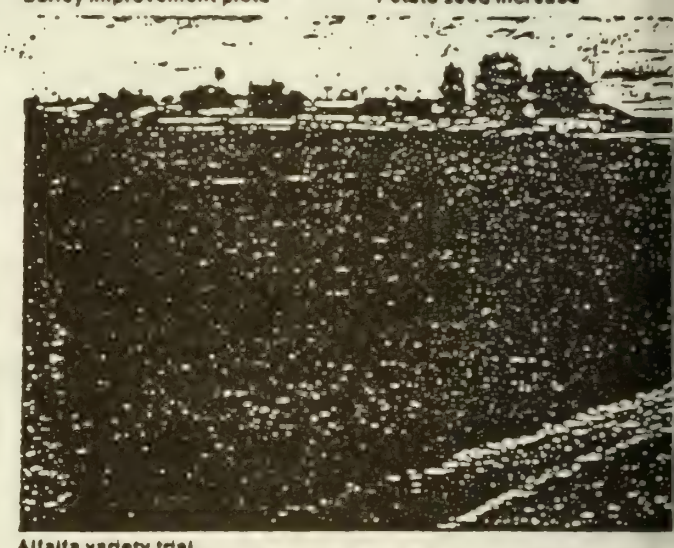
Potato seed increase

Tulelake

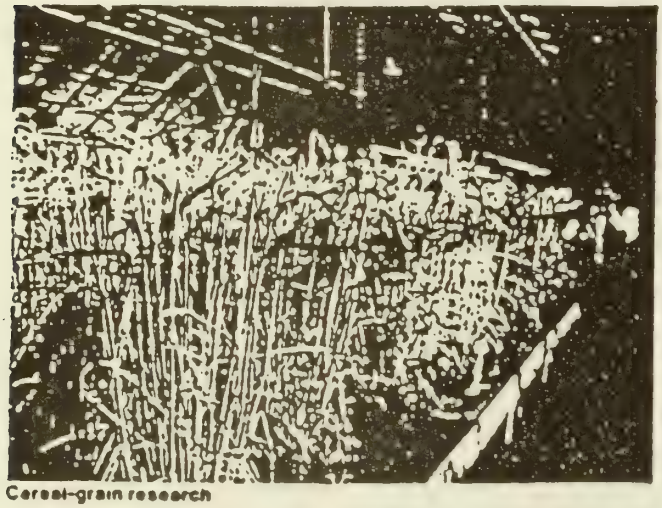
Located on a former lakebed near California's northern border, the Tulelake Field Station provides facilities for research on cool-climate field and vegetable crops, especially potatoes, onions, small cereal grains, and alfalfa — crops important to local farmers and the agribusiness community of the region.

Many new varieties of potatoes have been developed and evaluated there. Cereal grain breeding, genetic, and production research have been conducted at the station for many years. New and improved varieties of durum wheat, common wheat, feed barley, and triticale have been developed at Tulelake.

Nearly half of Tulelake's acreage is used in the development of crops particularly suited to northern California. Wild horseradish growing in the area was developed into a commercial crop at the station, and the region now accounts for a third of total U.S. production of this crop.



Alfalfa variety trial



Cereal-grain research

TULELAKE FIELD STATION
Highway 139 & Havallna Road
(P.O. Box 447)
Tulelake, CA 96134

(916) 667-5117

Research emphasis: Potatoes, sm
grains, pest management, onions

Size: 118 acres

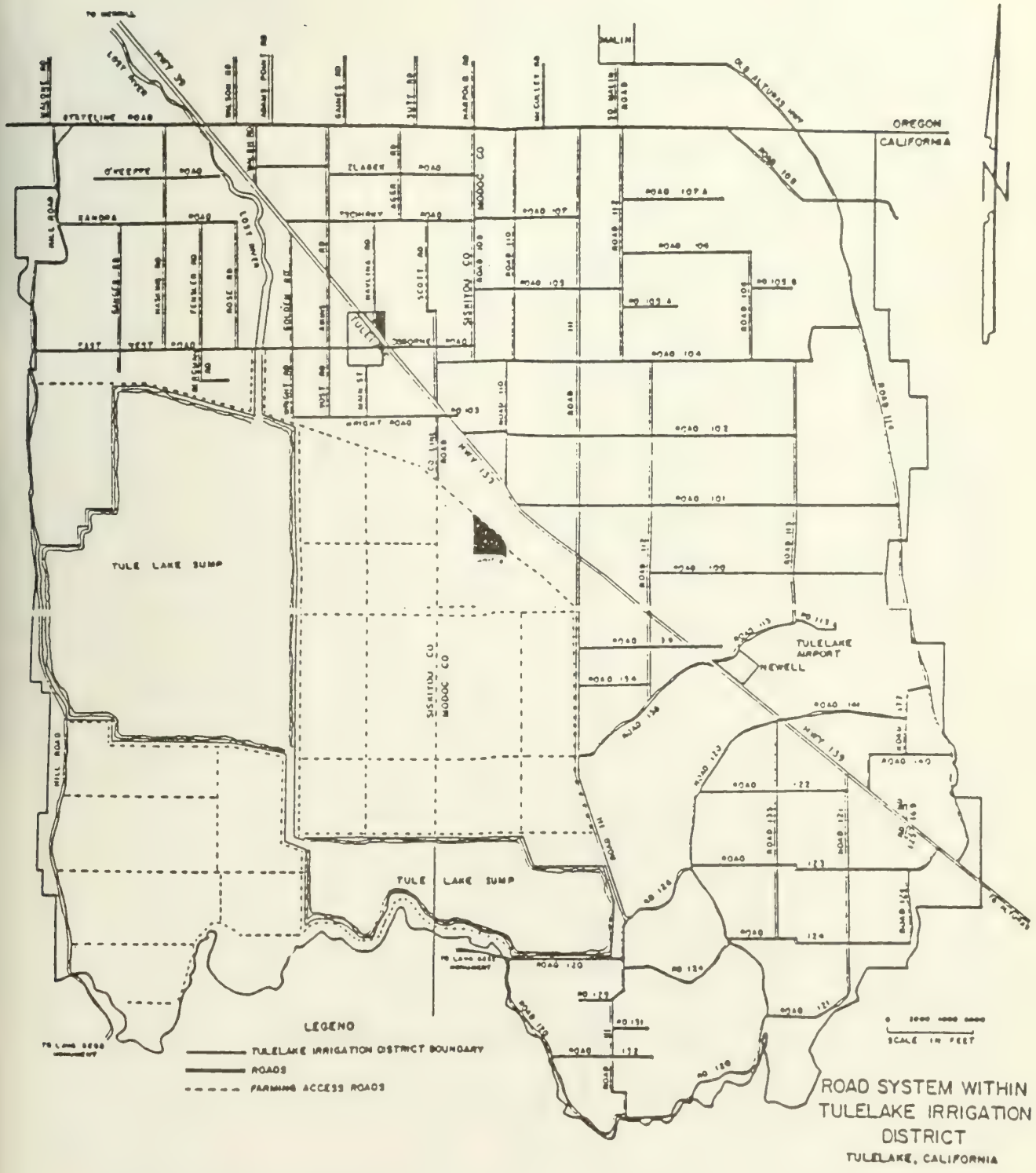
Climate zone: High mountain inter
valley

Terrain: Flat organic soils - former
lakebed

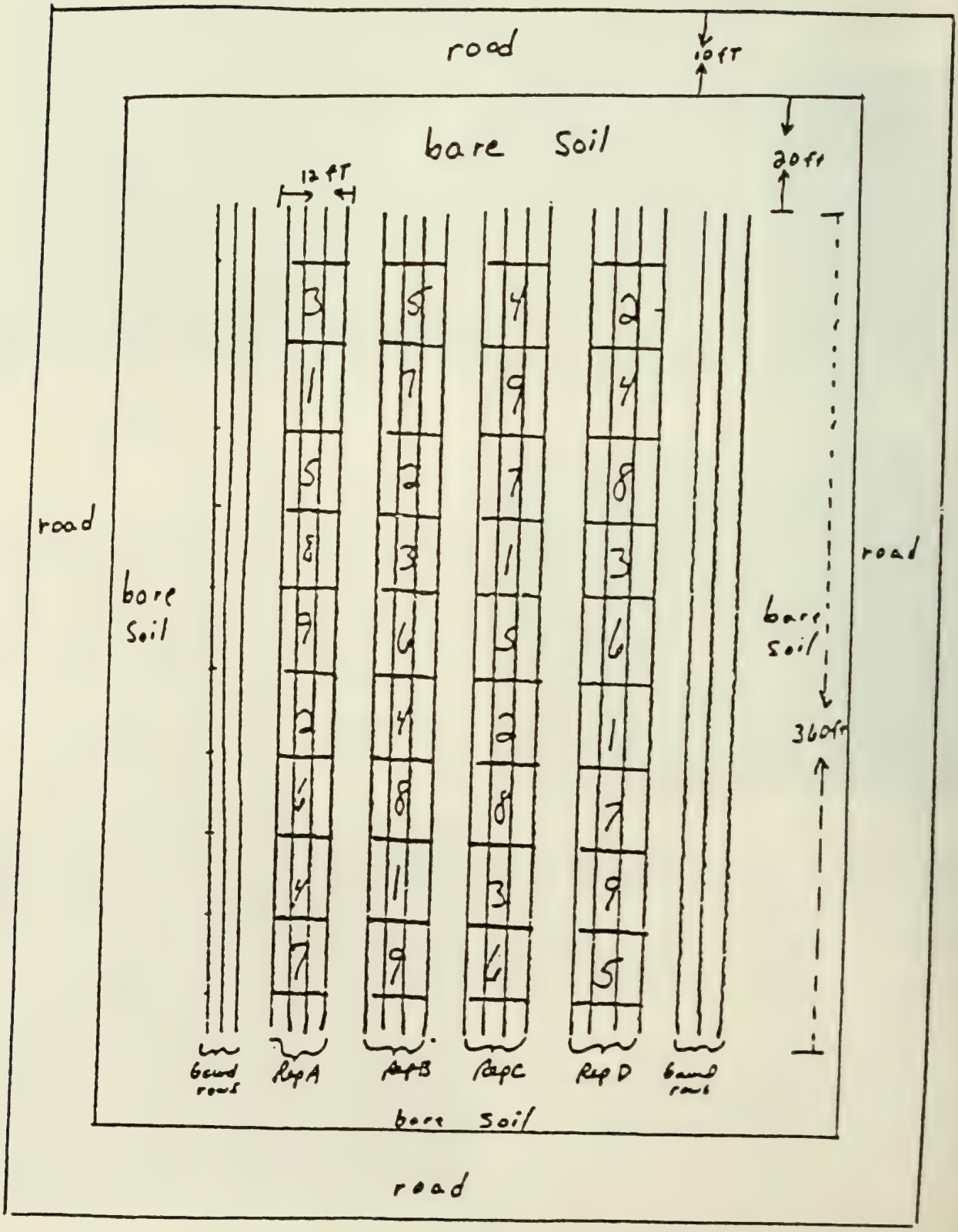
Average annual rainfall: 12.88 inch

Seasonal temperatures:
Annual mean: 49.7°F
Summer: Maximum monthly mean
73.0°F
Winter: Minimum monthly mean -
29.1°F

Elevation: 4,000 ft


OREGON
CALIFORNIA
TULE LAKE SUMP
TULE LAKE SUMP
SISKIYOU CO
MODOC CO
TULELAKE AIRPORT
NEWELL
LEGEND
TULELAKE IRRIGATION DISTRICT BOUNDARY
ROADS
FARMING ACCESS ROADS
SCALE IN FEET
ROAD SYSTEM WITHIN
TULELAKE IRRIGATION
DISTRICT
TULELAKE, CALIFORNIA

Appendix C, page 1



Appendix C, page 2

Treatments

1. Control, untreated during the entire growing season.

2. Plants sprayed at emergence with ice minus deletion mutants of strain TLP2.

3. Same as 2 except plants also sprayed with ice nucleation active TLP2.

4. Plants sprayed at emergence with ice minus deletion mutants of strain Cit 7.

5. Same as 4 except plants also sprayed with ice nucleation active Cit 7.

6. Plants sprayed at emergence with a mixture of ice nucleation active TLP-2 and Cit 7.

7. Plants sprayed at emergence with ice minus chemically mutant strain Cit 13-12.

8. Plants sprayed at emergence with a naturally occurring non ice nucleating strain of P. fluorescens. (A506)

9. Plants sprayed at emergence and at weekly intervals with the bactericide $Cu(OH)_2$.

Monday
April 15, 1985

Federal Register

Part II

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Availability of Environmental Assessment for Public Comment; Request for Comments on Need for a Programmatic Environmental Impact Statement; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Availability of Environmental Assessment for Public Comment; Request for Comments on Need for a Programmatic Environmental Impact Statement

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice.

SUMMARY: This notice announces the availability for public comment of the "Environmental Assessment and Finding of No Significant Impact," dated January 21, 1985, "concerning the application to the National Institutes of Health (NIH), under the NIH Guidelines for Research Involving Recombinant DNA Molecules, of Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damage to plants." In addition, this notice requests comments on the need for a programmatic Environmental Impact Statement in connection with NIH approvals of proposals to release into the environment organisms containing recombinant DNA molecules.

DATE: Comments must be received by May 15, 1985.

ADDRESS: The January 21, 1985, Environmental Assessment and Finding of No Significant Impact is available upon a request to the NIH Office of Recombinant DNA Activities (ORDA), Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland 20205. Phone: (301) 496–6051. Comments should be submitted to the same address and will be available for inspection and copying on weekdays between the hours of 8:30 a.m. and 5:00 p.m.

FOR FURTHER INFORMATION CONTACT: Dr. William J. Gartland, ORDA, Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland, 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION:

I. Background

The NIH Guidelines for Research Involving Recombinant DNA Molecules (49 FR 46266, November 23, 1984) require that recombinant DNA experiments "conducted at or sponsored by an institution that receives any support for recombinant DNA research from the National Institutes of Health" (section I-C of Guidelines) which involve "deliberate release into the environment of any organism containing recombinant DNA, except certain plants as described in Appendix L" (section III-A of Guidelines) must have prior approval from NIH before the experiment is conducted.

The NIH approved the application of Drs. Lindow and Panopoulous to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques on June 1, 1983 (48 FR 24549). Prior to the conduct of the field test, a lawsuit was filed challenging the approval on the grounds that NIH violated the National Environmental Policy Act (NEPA), (42 U.S.C. 4321 *et seq.*), and the Council on Environmental Quality (CEQ) regulations implementing NEPA (40 CFR Parts 1500–1508), by failing to prepare an Environmental Assessment (EA) or an Environmental Impact Statement (EIS) in connection with the approval of the field test.

On May 16, 1984, the United States District Court for the District of Columbia, in Civil Action No. 83–2714 (*Foundation on Economic Trends et al., v. Margaret M. Heckler et al.*), issued a preliminary injunction which enjoined NIH from "approving or continuing to approve experimentation involving the deliberate release of recombinant DNA under * * * the * * * NIH Guidelines * * * until such time as the Court enters final judgment." The University of California was enjoined from proceeding with the Lindow-Panopoulos experiment "until such time as the Court enters final judgment."

The order of the District Court was appealed by the NIH and the University of California and on February 27, 1985, the United States Court of Appeals for the District of Columbia vacated the District Court's injunction as it applied to future approvals of deliberate release experiments by NIH, but upheld the preliminary injunction as it applied to the University of California experiment, concluding that the injunction should remain in effect until completion of an appropriate Environmental Assessment.

II. The Environmental Assessment

The NIH had begun preparation of an Environmental Assessment of the University of California experiment after the entry of the District Court order and that assessment and a Finding of No Significant Impact (FONSI) were completed on January 21, 1985.

This notice announces the availability for public comment of this 64-page EA–FONSI.

For convenience, the Table of Contents of the EA–FONSI

Page

1.0 Introduction and Summary 1
2.0 Background: Previous Administrative Action 3
3.0 Purpose and Need 6
3.1 Purpose of the Proposed Action 8
3.2 Need for the Proposed Action 9
4.0 Description of Proposed Action and Alternatives 11
4.1 Proposed Action 11
4.2 Alternatives to the Proposed Action 16
4.2.1 Approval of Original Proposal (September 17, 1982) Without Restriction 16
4.2.2 Approval of Modified Proposal (March 8, 1983) Without Restriction 16
4.2.3 Approval of Proposal as Approved by NIH on June 1, 1983, With Changes Proposed by the Investigators 16
4.2.4 Withhold Approval 17
5.0 Environment Effects 17
5.1 The Physical Environment 17
5.2 Proposed Test Organisms 22
5.2.1 Characteristics of *P. s. syringae* and *E. herbicola* strains 22
5.2.2 Construction of INA Deletion Mutants 26
5.2.3 Previous Field Tests with INA Minus Bacteria 29
5.3 Effects of the Proposed Field Test 30
5.3.1 Risk Assessment 30
5.3.2 The Test Plot 34
5.3.3 Crops and Non-Commercial Plant Species 36
5.3.4 Local Epiphytic Bacterial Populations 37
5.3.5 Insect Populations 38
5.3.6 Animal Populations 41
5.3.7 Atmosphere and Climate 41
5.3.8 Scientific and Agricultural Benefits 43
5.3.9 Worst Case Considerations 44
5.4 Effects of Alternatives 44
5.4.1 Approval of Original Proposed Without Restriction 44
5.4.2 Approval of Modified Proposal Without Restriction 44
5.4.3 Approval of Proposal as Approved by NIH on June 1, 1983, With Changes Proposed by the Investigators 49
5.4.4 Withhold Approval 50
5.5 Summary and Conclusions 50
6.0 Consultation 52
7.0 References 53
Appendix A—Background Concerning the NIH Guidelines for Research Involving Recombinant DNA Molecules 57
Appendix B—Description and Location of Tulelake Field Station B1
Appendix C—Diagram of Test Plot and Description of Treatments C1

Comments on the EA and FONSI will be considered by the Director, NIH, when he decides whether to reapprove the field test proposed by the University of California.

III. The Need for A Programmatic Environmental Impact Statement

In its February 27, 1985, decision the Appeals Court stated that, "If NIH gives adequate environmental consideration to each deliberate release experiment . . . then we cannot, at this point, say that such consideration will obstruct environmental review. We thus reverse the District Court's finding that plaintiffs are likely to succeed in showing, at this point, that the governing law requires NIH to complete a programmatic EIS on deliberate release experiments." However, the Appeals Court went on to state that, "NIH should at least consider whether a programmatic EIS is required to fulfill the agency's legal obligations

under NEPA and the CEQ regulations." The NIH is initiating such a consideration and hereby requests comments on whether a programmatic EIS is required to fulfill NIH's obligations under NEPA and the CEQ regulations in connection with NIH approvals of proposals to release into the environment organisms containing recombinant DNA. The following information is provided to assist the public in commenting on this issue.

A. When is a Programmatic EIS Required?

In its decision of February 27, 1985, (p. 30 of the *Slip Opinion*) the Court of Appeals quoted a definition of a programmatic EIS from one of its previous decisions:

> A programmatic EIS reflects the broad environmental consequences attendant upon a wide-ranging federal program. The thesis underlying programmatic EISs is that a systematic program is likely to generate disparate yet related impacts. * * * Whereas the programmatic EIS looks ahead and assimilates 'broad issues' relevant to [the program], the site-specific EIS addresses more particularized considerations * * *. *Nat'l Wildlife Federation* v. *Appalachian Regional Comm'n*, * * * 677 F.2d at 888.

The Court of Appeals also referred (pp. 30–31 of the *Slip Opinion*) to criteria for determining when a programmatic EIS should be prepared:

> Under CEQ regulations a programmatic EIS should be prepared if actions are 'connected,' 'cumulative,' or sufficiently 'similar' that a programmatic EIS is 'the best way' to identify the environmental effects. 40 CFR § 1508.25. As this court has noted, 'Two considerations are especially helpful in reviewing the responsible officials' decision not to prepare a programmatic EIS: (a) Could the programmatic EIS be sufficiently forward looking to contribute to the decisionmakers' basic planning of the overall program? and (b) Does the decisionmaker purport to 'segment' the overall program, thereby unreasonably constricting the scope of * * * environmental evaluation?' *Nat'l Wildlife Federation* v. *Appalachian Regional Comm'n*, * * * 677 F.2d at 889. Thus a programmatic EIS should be prepared if it can be forward-looking and if its absence will obstruct environmental review.

B. Field Tests Approved by NIH

To date, NIH has approved three field tests of organisms containing recombinant DNA. One involved tomato and tobacco plants. When this was reviewed by the NIH Recombinant DNA Advisory Committee (RAC) on October 25, 1982, the statement was made that "the probability that the experiment would be successful is very low." In fact, the researcher found that the experiment did not succeed under laboratory conditions, and therefore for scientific reasons he does not wish to proceed with the field test.

This leaves only two cases of NIH approval for field tests of organisms containing recombinant DNA in which the investigators wish to proceed. One is the Lindow-Panopoulos experiment involving ice-nucleation-minus bacteria on which the January 21, 1985, EA–FONSI was prepared. The other, from Dr. Ronald Davis of Stanford University, involves corn plants (*Zea mays*) transformed with corn DNA. The environmental concerns raised by these two proposals are totally different and can only be meaningfully considered individually through an evaluation of the specific details of the proposed field test and the characteristics of the organisms proposed to be released, including their ecological niche, pathogenicity, viability and ability to escape from the field test site.

C. NIH's Role in the Approval or Disapproval of Proposals for the Release into the Environment of Organisms Containing Recombinant DNA

The Federal action for purposes of NEPA and the CEQ regulations is reviewing proposals for deliberate release experiments submitted to NIH by institutions which receive NIH funds for recombinant DNA research. There is no Federal program for the planning, conduct, and support of deliberate release experiments; there is only a Federal requirement that deliberate release experiments by NIH-funded institutions be approved by NIH.

This Federal requirement does not involve federally planned or initiated actions that are directed toward a particular objective. Proposals are submitted by different researchers from different fields at undetermined times for quite different experiments. Of the three proposals approved to date, two were not funded by NIH. They were submitted for approval because the institutions proposing to conduct the field tests received some NIH funds for recombinant DNA research.

The NIH's Guidelines are not "aimed at developing new technology which, when applied, will affect the environment." Rather, the Guidelines are aimed at scrutinizing the development of technology to ensure the safety of that development. In the course of this scrutiny of proposals for the field testing of organisms containing recombinant DNA, NIH has not identified any common environmental effect which could arise for the field tests that have been approved by NIH thus far.

The NIH does not know what proposals for deliberate release experiments may be submitted for approval in the future since most of the experiments are investigator initiated and are not planned by NIH.

D. NIH's Future Role in the Review and Approval of Proposals for the Deliberate Release Into the Environment of Organisms Containing Recombinant DNA

Until recently, the NIH was the only Federal agency reviewing and approving proposals involving the deliberate release into the environment of organisms containing recombinant DNA. That is no longer the case.

(1) On October 17, 1984, the Environmental Protection Agency (EPA) published in the Federal Register (49 FR 40659) and Interim Policy on Small Scale Field Testing of Microbial Pesticides which requires notification to EPA of proposed field tests involving certain microbial pesticides after which EPA will determine whether an Experimental Use Permit is required.

Advance Genetic Sciences, Inc. (AGS), had previously voluntarily submitted a proposal for NIH review and approval dealing with a field test of *Pseudomonas* strains containing recombinant DNA. The NIH gave notice of the upcoming review (49 FR 17672, April 24, 1984), and the proposal was considered at the June 1, 1984, RAC meeting. The NIH still had the proposal under review when AGS in a letter of February 11, 1985, withdrew the proposal from the NIH review process, deciding to rely only on EPA review under the October 17, 1984, Federal Register notice.

(2) On December 31, 1984, the Office of Science and Technology Policy published in the Federal Register (49 FR 50856) for public comment a Proposal for a Coordinated Framework for Regulation of Biotechnology. It includes a proposal to establish agency-based scientific advisory committees modelled after the NIH RAC in four other agencies (EPA, the Food and Drug Administration (FDA), the Department of Agriculture (USDA) and the National Science Foundation (NSF)).

If this new scientific advisory structure is established, proposals for deliberate release of organisms containing recombinant DNA that would have previously come to NIH for review and approval may go instead to EPA, USDA or NSF.

Dated: April 3, 1985.

James B. Wyngaarden,

Director, National Institutes of Health.

[OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official Government programs contained in the Catalog of Federal Domestic Assistance. Normally, NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program, but also essentially every Federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every Federal program would be included as many Federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the Catalog of Federal Domestic Assistance are affected]

[FR Doc. 85-8798 Filed 4-12-85; 8:45 am]

BILLING CODE 4140-01-M

PUBLIC AND SCIENTIFIC AFFAIRS BOARD

AMERICAN SOCIETY FOR MICROBIOLOGY

1913 I Street, N.W.
Washington, D.C. 20006
Telephone: (202) 822-9229

April 24, 1985

Dr. William Gartland
NIH Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, MD 20205

Dear Dr. Gartland:

The Public and Scientific Affairs Board (PSAB) of the American Society for Microbiology (ASM) is pleased to respond to the FEDERAL REGISTER notice of April 15, 1985 (50 FR: 14794-14796) concerning the "Environmental Assessment and Finding of No Significant Impact" (EA-FONSI) for the application to the National Institutes of Health (NIH) under the NIH Guidelines for Research Involving Recombinant DNA Molecules, of Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damage to plants. The ASM is the largest single biological life sciences organization in the world with an active membership of over 31,000. The PSAB wishes to comment on the EA-FONSI for the Berkeley experiment and the need for a programmatic Environmental Impact Statement (EIS) in connection with NIH approval of proposals to release into the environment organisms containing recombinant DNA molecules.

The Environmental Assessment

The Environmental Assessment (EA) prepared by the NIH in relation to the experiments proposed by the Berkeley scientists is exhaustive in its coverage and persuasive in its arguments. It describes all proposed actions in detail and thoughtfully identifies and then considers the environmental effects of risks posed by those actions. We can think of no points to consider that are not addressed by the document. The agricultural history of the site is summarized. Climatological data is presented, and the animal and the human populations surrounding the experimental site have been surveyed. Reasonable consideration also was given to the probability of escape from the test site, to transport, and to survival of the organism after escape. Finally, although not required, the EA concludes with worst case considerations that describe what is remotely or infinitesimally possible, but highly unlikely.

We concur in the opinion expressed in the EA that the proposed studies by the Berkeley scientists will have no significant impact on the environment for the following reasons: 1) No functional gene has been added to the bacterium. Rather, a single bit of chromosomal information has been biochemically deleted from the bacteria that will be applied to the test site; 2) Mutation of the same

organism has been accomplished previously using techniques other than recombinant DNA, and deletion mutants have been demonstrated to occur naturally. Therefore, the organism produced by recombinant DNA deletion cannot be considered unique; and 3) Since the test organism does not contain a foreign gene and is not unique, it poses little or no added risk than that experienced under what are considered natural conditions.

The EA makes a convincing case for the need for the proposed field test. We are certainly persuaded that the benefit of using ice-nucleation-minus bacteria will justify any risk that may be involved. Alternative procedures for protecting plants against frost damage require large amounts of energy and water or consume large quantities of fossil fuels, and they can create health hazards and adversely affect environmental quality. The Berkeley case offers certain benefits and limited risk, if any risk at all.

The Need for a Programmatic Environmental Impact Statement

We question whether an EIS is desirable or necessary in the specific case of the experiment by Drs. Lindow and Panopoulos to test ice-nucleation-minus bacteria prepared by recombinant-DNA techniques. We also question whether a programmatic EIS should be required in any case where an adequate EA is provided. By definition, a programmatic EIS is concerned with broad issues rather than with specific considerations which relate to a particular site. In the Berkeley case, and it seems to us in all but the most unusual circumstance, an EA will serve as well as an EIS to identify environmental effects. The EA is more concerned than the EIS with the present. The EIS is more forward-looking than the EA, and the availability of an EA without an EIS will not necessarily obstruct environmental review. The absence of a programmatic EIS does not hinder a continuing concern for environmental effects.

The thoroughness of preparation of the EA and the fact that the organism to be tested by the Berkeley scientists does not contain a foreign gene and is not unique, obviates the need in this case for a programmatic EIS. If future EAs are as thoroughly and well prepared as the present document, there should be little need in general for programmatic EISs. Nevertheless, any experiment which proposes to take an organism from the laboratory and apply it in the field must undergo careful review, and the need for that review must be determined on a case-by-case basis. Given an EA, the decision concerning need for a programmatic EIS also should be made on a case-by-case basis. Every effort should be made to see that a programmatic EIS is not required if all it accomplishes is to duplicate what is already presented in an EA.

The PSAB applauds the NIH and the NIH Recombinant DNA Advisory Committee (RAC) for the way they have managed a difficult assignment very well. In conclusion, we believe that NIH has effectively dealt with this issue and that there is no further need for a programmatic Environmental Impact Statement. The NIH has acted cautiously while at the same time untold financial damage was incurred this past year in Florida. In spite of a careful and cautious review of all

aspects of the Berkeley experiment, a significant delay has occurred in the transfer of research from the laboratory to desirable and appropriate field tests. We believe that the results of this experiment may ultimately contribute to human well-being by alleviating hunger here and elsewhere in the world. The PSAB is pursuaded that the field trials should be allowed to proceed without further administrative delay.

Sincerely,

Harlyn O. Halvorson

Harlyn O. Halvorson, Ph.D.
Chairman, Public and Scientific Affairs Board

David Pramer

David Pramer, Ph.D.
Chairman, Committee on Environmental Microbiology

Monica Riley

Monica Riley, Ph.D.
Chairman, Committee on Genetic and Molecular Microbiology

Kenneth Berns

Kenneth I. Berns, M.D., Ph.D.
Chairman, Committee on Medical Microbiology and Immunology

PURDUE UNIVERSITY SCHOOL OF VETERINARY MEDICINE

May 3, 1985

Dr. William Gartland
ORDA, Bldg 31 Rm 3B10
NIH, USPHS, HHS
Bethesda, MD 20205

Dear Dr. Gartland:

This is to support and commend the statement made by the Public and Scientific Affairs Board of the American Society for Microbiology regarding the 64 page EA-FONSI related to the NIH approval of the application of Drs. Lindow and Panopoulous to field test ice-nucleation - minus bacteria prepared by recombinant DNA techniques (48FR24549).

The statement is intelligent, concerned and powerful. It is from responsible, well informed scientists. I add anothervoice in support to the end that this work and more like it in the future be freed from such mischievous interference. I am a member of the American Society for Microbiology, the American Society for Virology, the American Association of Immunologists, the American Association for the Advancement of Science and a fellow of the American Academy of Microbiology.

Yours very truly,

Donald P. Gustafson

Donald P. Gustafson, DVM,PhD
Professor of Virology

DPG:lh

DEPARTMENT OF VETERINARY MICROBIOLOGY
PATHOLOGY AND PUBLIC HEALTH
Veterinary Pathology Building
West Lafayette, Indiana 47907

Agracetus
"A Partnership of Cetus Corporation and W.R. Grace & Co."

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April 24, 1985

Dr. William J. Gartland, Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20205

Dear Dr. Gartland:

This letter is in response to the April 15 Federal Register notice inviting comment on: 1) the "Environmental Assessment and Finding of No Significant Impact" for the application to field test ice-nucleation-minus bacteria, 2) the need for a Programmatic Environmental Impact Statement.

Environmental Assessment and Finding of No Significant Impact

This document appears to be a thorough, balanced and accurate assessment of the environmental effects of conducting the proposed field test of ice-nucleation-minus bacteria produced through recombinant DNA technology. Clearly a tremendous amount of thought and work has gone into its preparation. No documenation approaching this magnitude has previously been required for introduction to the environment of any microorganism, considered to be safe, that contains a man-made genetic alteration.

Many individuals with diverse experience in areas relevant to the proposed project have evaluated potential environmental consequences of this experiment and have concluded that the chance of damage is sufficiently low that the experiment should no longer be delayed. The Environmental Assessment and Finding of No Significant Impact clearly indicates to the public that there is no cause for concern. The consultants identified at the end of the assessment include scientists who are well-versed with the issues and who have nothing personal to gain from the outcome of the experiments. These scientists are internationally recognized for their scientific judgments.

Thousands of different mutant microorganisms have been released to the environment over a period of half a century. It is important to realize that there is no confirmed case of environmental damage caused by release of a safe microorganism that has been genetically modified in the laboratory. The Environmental Assessment strongly supports the conclusion that this experiment will not affect the environment in any substantively different way than the thousands of "experiments" involving mutants of safe microorganisms that have already proceeded without problems.

The Need For a Programmatic Environmental Impact Statement

Man has had centuries of experience with plant breeding, a century of experience with genetically altering microorganisms, and half a century of experience with gene exchanges among microorganisms. No special concerns have been raised from release of organisms produced through man-made and man-selected genetic alterations. We consider these types of projects to be very safe; consequently, we have no regulations to govern release of genetically altered organisms which are regarded as safe.

In comparison to traditional genetic technologies, recombinant DNA technology actually is more precise and predictable in its outcome. Thus, the level of concern about problems from recombinant DNA techniques should be no more than the level of concern associated with release of organisms developed using conventional techniques.

The NIH should not prepare a Programmatic Environmental-Impact Statement unless there is a scientifically sound reason why application of recombinant DNA technology will inadvertantly create a serious environmental problem. The fact that a Programmatic Environmental Impact Statement has never been deemed necessary for traditional practices such as breeding and mutation, strongly suggests that a Programmatic Environmental Impact Statement would not be useful for recombinant DNA work. Arguments must be made by proponents of a Programmatic Environmental Impact Statement to demonstrate that a recombinant organism indeed has a greater chance to create environmental damage than a genetically altered organism derived through traditional practices.

I appreciate this opportunity to comment on these important matters and hope that the procedural complexities that have diverted attention from moving this science ahead will soon be overcome.

Sincerely,

AGRACETUS

Winston J. Brill, Ph.D.
Vice President, Research and Development

WJB:es

association of american medical colleges
One Dupnt Circle, N.W., Washington, D.C. 20036/(202) 828-0400

May 7, 1985

Dr. William J. Gartland
ORDA
Building 31, Room 3B10
National Institutes of Health
Bethesda, MD 20205

Dear Dr. Gartland:

The Association of American Medical Colleges (AAMC) is pleased to respond to the FEDERAL REGISTER notice of April 15, 1985 (50 FR:14794-14796) concerning the Environmental Assessment, in connection with the application of Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques.

The Environmental Assessment

The Environmental Assessment prepared by the NIH in relation to the proposed Lindow/Panopoulos experiment is detailed and thorough. The National Institutes of Health and the NIH RAC are to be congratulated for establishing the procedures necessary to ensure proper review of proposed applications to conduct research which involve field testing or deliberate release into the environment of genetically engineered organisms. The establishment of the Working Group on Environmental Release of the Recombinant DNA Advisory Committee and the decision to prepare an Environmental Assessment statement on each such proposed experiment for deliberate release are both laudable parts of this process.

The Need for a Programmatic Environmental Impact Statement

The FEDERAL REGISTER notice solicits opinion about whether a programmatic Environmental Impact Statement (EIS) is necessary in the case of this experiment. It would certainly seem that the Environmental Assessment (EA) statement prepared to accompany the application of Lindow/Panopoulos is thorough enough to permit a full judgment of the environmental impact of their proposed experiment. Since it concludes that there is no significant environmental impact, a further Environmental Impact Statement is not also necessary in this case.

The Association strongly supports the argument advanced by the NIH in the FEDERAL REGISTER that a programmatic EIS is neither necessary nor appropriate for future research proposals involving field trials of genetically engineered microorganisms. A single a priori programmatic EIS cannot be made in regard to such experiments. They are not part of a deliberate NIH research program but rather unsolicited and as yet unknown proposed future experiments. Each experiment will be a unique instance which must be evaluated for environmental impact on a case-by-case basis. The Environmental Assessment (EA) statement admirably serves this purpose

by providing a full evaluation of the environmental impact of a given proposed experiment involving field testing of a genetically engineered organism. If an EA were to conclude that significant environmental impact was anticipated, the NIH could then prepare an individual EIS for that proposed experiment.

Sincerely,

John F. Sherman, Ph.D.
Vice President

Zoecon Corporation
A Sandoz Company
975 California Avenue, Palo Alto, California 94304-0859
P.O. Box 10975, Palo Alto, California 94303 (415) 857-1130

May 8, 1985

N.I.H. Office of Recombinant DNA Activities (ORDA)
Building 31 RM. 3B10
National Institutes of Health
Bethesda, MD 20205

Subject: Research Involving r DNA Molecules by Drs. Steven Lindow and Nickolas Panopoulos: Environmental Assessment and Finding of No Significant Impact.

Dear Sirs:

Zoecon Corporation, A Sandoz Company is pleased to submit these comments on the subject EA and FONSI. Zoecon is a pioneer in the development of so called 'biorational' pest control agents and is one of the larger producers of both biochemical and microbial pesticides.

Zoecon agrees with NIH's Comprehensive Environmental Assessment and finding of no significant impact with respect to the testing proposed by Drs. Lindow and Panopoulos. The facts that: (a) the INA$^-$ deletion mutant of the Pseudomonas syringae pv. syringae is isogenic with the naturally occurring P. syringae INA$^+$ and contains no foreign DNA; and (b) that non isogenic INA$^-$ mutants produced through undirected mutagensis have been previously tested without adverse environmental effects; argue convincingly that the risk associated with the proposed field use is negligible. Also there is a finite probability that such isogenic deletion mutants have occured naturally in the past without notable effect and that the persistance and competitiveness of INA$^-$ mutants in general is insufficient to allow them to displace the wild type whose INA$^+$ character probably confers a distinct competitive advantage for epiphytic colonization.

With respect to the need for a Programmatic Environmental Impact Statement, Zoecon believes that in view of the increasing responsibilities of other Federal agencies notably the Environmental Protection Agency, such programmatic EIS should not be required of N.I.H. It seems likely that future reviews by ORDA will focus, as in the past, on laboratory hazards rather than specifically on environmental impact. It is hoped however, that sufficient cooperation will

exist between the involved agencies that ORDA will be able to provide helpful advice from its longer experience base.

Sincerely,

Joseph P. Brown

Joseph P. Brown, Ph.D.
Director, Toxicology & Regulatory Affairs

cc: E.C. Spurrier (NACA)

(0820m)

May 10, 1985

NIH Office of Recombinant DNA Activities(ORDA)
Building 31
Room 3B10
National Institutes of Health
Bethesda, MD 20205

RE: Federal Register Notice of April 15, 1985

Genencor, Inc., an industrial enzyme producer, would like to take this opportunity to comment on the above referenced Federal Register notice requesting comment on the "Environmental Assessment and Finding of No Significant Impact" dated January 21, 1985 "concerning the application to the National Institutes of Health (NIH), under the NIH Guidelines for Research Involving Recombinant DNA Molecules, of Drs. Steven Lindow and Nickolas Panopolous of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damage to plants."

We have reviewed the Environmental Impact Statement (EIS) and find it to be thorough and well done and we agree with the finding by NIH of no significant impact. Further, from our understanding of the programatic EIS and the varied nature of recombinant organisms and environments in which they may be released, we feel that the assessment of their environmental impact can best be determined on a case-by-case basis through site specific environmental impact statements rather than a programatic EIS.

We thank you for this opportunity to comment.

Sincerely,

Alice K. Jameson

Alice K. Jameson
Manager of Regulatory Affairs

Southwest Foundation
for Biomedical Research

West Loop 410 at Military Drive
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San Antonio, Texas 78284
(512) 674-1410

May 9, 1985

NIH Office of Recombinant DNA Activities
Building 31
Room 3B10
National Institutes of Health
Bethesda, MD 20205

Re: Comment on the Need for a Programmatic Environmental Impact Statement (EIS)

The following comments are the opinion of the individual scientist whose signature is included and may not reflect the opinion of other scientists or individuals at the Foundation at which the scientist is employed.

It appears that the National Institutes of Health in initially reviewing the application of Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques and in issuing an Environmental Assessment and a Finding of No Significant Impact has fulfilled its mission. The preparation of a programmatic Environment Impact Statement (EIS) seems unnecessary, particularly as its absence would not appear to obstruct environmental review by other agencies. An EIS prepared by NIH could be redundant because of a Proposal for a Coordinated Framework for Regulation of Biotechnology proposed by the Office of Science and Technology Policy (Federal Register 49 FR 50856). Should such an inter-agency advisory structure be established, it might appropriately review proposals for deliberate release of organisms containing recombinant DNA and conceivably issue a programmatic EIS for this particular field test experiment.

C. Elizabeth Castro

C. Elizabeth Castro, Ph.D.

University of
Nebraska
Lincoln
Institute of Agriculture and Natural Resources

Department of Plant Pathology
406 Plant Sciences Hall
East Campus
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May 13, 1985

Dr. William J. Gartland, Jr.
National Institutes of Health
Building 31, Room 3B10
Bethesda, MD 20205

Dear Dr. Gartland:

Relative to the Environmental Assessment for the proposal to field test ice-nucleation-minus bacteria, I find the document to be appropriate and comprehensive. I do not see a need for a programmatic Environmental Impact Statement since anticipated proposals and actions by RAC are not connected, cumulative or likely to be sufficiently similar, criteria which I understand are considered standard for such an EIS.

I do have comments on the accuracy of the EA document, which could affect the interpretation of certain parts.

Page 22. 5.2.1.A.1. Nomenclature & taxonomy. Pseudomonas syringae is not an 'abbreviated taxonomic designation of Pseudomonas syringae pathovar (pv.) syringae 'see e.g. Bergey's Manual of Systematic Bacteriology, Vol. 1. 1984, p. 168). Rather, P. syringae is a taxon with nomenclatural status according to the International Code of Nomenclature of Bacteria (1976); infrasubspecific taxa, such as pathovars, do not have such a status and are not part of the taxonomic hierarchy. The term pathovar is used to refer to a strain or set of strains with the same or similar characteristics, differentiated at the infrasubspecific level from other strains of the same species or subspecifies on the basis of distinctive pathogenicity to one or more plant hosts (Dye et al. 1980; Rev. Pl. Pathology 59:153-168). That is, it makes no sense and is confusing to create pathovar names for sub-populations of species where there is no evidence that they have any pathogenic character. Thus, the epithet P. syringae is correct, since the strains being worked with are not and never have been shown to be pathogenic. An example of such correct usage is found in Gross, D. et al., 1983, Appl. Evn. Microbiol. 46:1370-1379. Those strains of P. syringae that have been shown to be pathogenic may warrant the designation of pv. syringae or other pathovar epithet.

Page 23. L.1. P. syringae and P. syringae pathovars are common plant epiphytes. None of these taxa has ever been shown.... and do not grow...

Dr. William J. Gartland, Jr.
Page 2
May 13, 1985

Paragraph two: L. 1. Certain strains or isolates of a pathovar of *P. syringae* (*P. s.* pv. *syringae*) have been...

Page 24. Last paragraph: In summary, there are both non-pathogenic and pathogenic strains of *P. syringae*. Some strains of *P. s.* pv. *syringae* have...(This is analogous, for example, to the situation of the well-known *Escherichia coli*, which consists of both pathogenic and non-pathogenic strains. However, pathogenic strains of this bacterium are not given a latinized infrasubspecific epithet).

Page 25. L. 3. *P. syringae* colonizes the leaf surfaces of different plants; some of these strains seem to be specific for certain host plants.

Page 33. Paragraph one. It should not be implied that all naturally-occurring *P. syringae* have arisen due to deletion mutations. There is no evidence for this that I know of; i.e. it is likely that many, if not most, INA-*P. syringae* never were INA^+ (e.g. see Gross, reference above).

I hope these comments are helpful.

Sincerely,

Anne K. Vidaver
Professor & Head

IBA

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Industrial Biotechnology Association
2115 East Jefferson Street
Rockville, Maryland 20852
Telephone: (301) 984-9598

Harvey S. Price
Executive Director

May 14, 1985

Dr. William J. Gartland
Office of Recombinant DNA
Activities
Building 31, Room 3B10
National Institute of Health
Bethesda, Maryland 20205

Re: Environmental Assessment and
Finding of No Significant
Impact (January 21, 1985)

Dear Dr. Gartland:

The Industrial Biotechnology Association would like to express its agreement with the conclusion of NIH's environmental assessment in the ice-nucleation experiment case, that the modified field test proposal would have no significant environmental impact. We believe that the formal assessment makes clear that NIH's conclusion is supported by careful thought and abundant scientific evidence. While mindful of other public interests, the interest of encouraging such promising biotechnology research argues that NIH's substantial and conscientious effort to date should now be judged as fully adequate.

We also concur in NIH's earlier conclusion that a programmatic environmental impact statement would not be appropriate in connection with environmental release approvals at this time, for the facts and reasons set out in item III of the Federal Register notice.

Sincerely,

Harvey Price

IBA

MEMBER COMPANIES
INDUSTRIAL BIOTECHNOLOGY ASSOCIATION

AGRIGENETICS CORPORATION
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CETUS CORPORATION
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S. San Francisco, California

GENETICS INSTITUTE
Cambridge, Massachusetts

GENEX CORPORATION
Rockville, Maryland

GIST-BROCADES USA INC.
Charlotte, North Carolina

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Nutley, New Jersey

IMMUNEX CORPORATION
Seattle, Washington

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New Brunswick, New Jersey

LIFECODES CORPORATION
Elmsford, New York

MELOY LABORATORIES, INC.
Revlon Health Care Group
Springfield, Virginia

MOLECULAR GENETICS INC.
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MONSANTO COMPANY
St. Louis, Missouri

MYCOGEN CORPORATION
San Diego, California

PHARMACIA INC.
Piscataway, New Jersey

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SANDOZ, INC.
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SCHERING-PLOUGH CORPORATION
Madison, New Jersey

G.D. SEARLE & COMPANY
Chicago, Illinois

SHELL OIL COMPANY
Houston, Texas

SMITH KLINE & FRENCH LABORATORIES
Philadelphia, Pennsylvania

STANDARD OIL COMPANY (Indiana)
Naperville, Illinois

TRANSGENE
Paris
France

THE UPJOHN COMPANY
Kalamazoo, Michigan

Lilly Research Laboratories
A Division of Eli Lilly and Company
307 East McCarty Street
Indianapolis, Indiana 46285

Irving S. Johnson, Ph.D
Vice President

May 14, 1985

Dr. William J. Gartland, Jr.
Director, Office of Recombinant
DNA Activities
National Institute of Allergy
and Infectious Diseases, 31/3B10
National Institutes of Health
Bethesda, Maryland 20205

Dear Dr. Gartland:

We are replying to the request for comments on the Environmental Assessment (EA) prepared by the National Institutes of Health (NIH) on the experiments in the "Environmental Assessment and Finding of No Significant Impact" (EA-FONSI) for the application to the NIH under the NIH Guidelines for Research Involving Recombinant DNA Molecules, of Drs. Steven Lindow and Nickolas Panopoulos, University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damage to plants. The specific question for which comments are requested concerns the need for a programmatic Environmental Impact Statement (EIS) relative to NIH approvals of such experiments. We offer the following statement regarding the rational absence of such a need:

> We certainly question whether an EIS is desirable or necessary in this case. We also question whether a programmatic EIS should be required in any case where an adequate EA is provided. By definition, a programmatic EIS is concerned with broad issues rather than with specific considerations which relate to a particular site. In these experiments and, it seems to us in all but the most unusual circumstances, an EA will serve as well as an EIS to identify environmental effects. The EA is more concerned than the EIS with the present.
>
> To suggest that a programmatic environmental impact statement be developed for recombinant DNA technology after two or three experiments involving field tests of

recombinant organisms have been described is at best premature. It is a bit like asking for a description of the long-term effects of the discovery of x-rays on our civilization immediately after Röntgen showed the invisible radiation would penetrate some objects and not others and that these effects could be measured. Science deals with limited extrapolation from known experimental results; the data from one experiment must be digested and interpreted before the next can begin.

There can never be an original experiment with zero risk. With admirable caution, the NIH-Recombinant DNA Advisory Committee (RAC) has recommended approval of experiments that carefully probe new areas of application of recombinant DNA technology. The field test proposal of Drs. Lindow and Panopoulos is such an experiment. The environmental assessment prepared by NIH summarizes current knowledge about all factors bearing on possible environmental effects; we concur with the logical conclusion that no significant environmental impact should result. The EPA has requested additional information from the submitters before issuing an Experimental Use Permit (EUP) [the agency decided an EUP would be required]; however, these do not appear to be aimed at any previously unknown facts about the organism or the experimental conditions that would suggest that the environmental assessment was in error. What this action does suggest, however, is that experimental field tests and other experiments involving recombinant organisms may require the collection, assembly, and submission of an inordinate amount of information in defense of protocols that, in many instances, simply duplicate in a more refined way the processes of genetic recombination that exist naturally in the biosphere.

It is appropriate to begin consideration of certain categories of recombinant DNA experiments involving release of innocuously different organisms (in terms of their defined genomic changes) as being exempt from these requirements for extensive background information based on the fact that such changes occur naturally--and often with less precision. Of course, there are experiments that should not be classed as exempt at this stage, e.g.,

those in which the recombinant methodology involves expression of entirely new substances or eliminates a unique control susceptibility without providing an alternative. However, recombinant systems--as in the ice-nucleation experiments--that simply accomplish through a different mechanism what occurs in nature cannot be viewed as unique. It is very doubtful that any test method of examination of the altered organism could distinquish which pathway was employed in achieving the change. In particular, mechanism changes in the genome of an organism which can be documented as occurring naturally such as spontaneous deletions, point mutations, transversions, transpositions, translocations, and insertions might be considered an exempt category. Also included could be self-cloning experiments, those in which the organisms can be shown not to survive beyond one season in the environment, and those which simply provide increased amounts of essential amino acids or other simple nutrients.

The question of possible occupation of an unusual ecological niche is not answered through such action; the fact to be realized, however, is that the same unanswered questions exist for organisms undergoing mechanistically natural changes.

These are not uniquely the province of recombinant DNA technology. New ecological concerns are constantly being raised in a biologically dynamic world. The advisory role of the RAC is properly played when it provides recommendations bearing on the applications of recombinant technology in previously unprecedented situations. The technological fix of a technological error is no worse than the technological fix of a natural error; the natural fix of a natural or technological error (a new environmental equilibrium) is frequently not in the interest of humanity. The important feature in an environmental release experiment where uncertainties about occupation of a new ecological niche are great is that there is retention of or insertion of a control susceptibility, e.g., chemical sensitivity, sterility, essential nutrient growth requirement.

We commend NIH and RAC for the way they have managed a difficult assignment very well. In conclusion, we believe NIH has effectively dealt with this issue and there is no further need for a programmatic EIS. The NIH has acted cautiously, but at the same time untold financial damage was incurred this past year in Florida due to the freezing of crops. In spite of a careful and cautious review of all aspects of the Berkeley experiment, a significant delay has occurred in the transfer of research from the laboratory to desirable and appropriate field tests. We believe the results of this experiment may ultimately contribute to human well-being by alleviating hunger here and elsewhere in the world. We feel the field trials should be allowed to proceed without further administrative delay.

Very truly yours,

Irving S. Johnson

ISJ/as/rl

NATIONAL AGRICULTURAL CHEMICALS ASSOCIATION
THE MADISON BUILDING
1155 Fifteenth Street, N.W., Washington, D. C. 20005
202 • 296-1585 Cable: NAGACHEM

Dr. Earl C. Spurrier
Vice President
Regulatory Affairs

May 15, 1985

Dr. William J. Gartland
Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20205

Dear Dr. Gartland:

The following statements from the National Agricultural Chemicals Association (NACA)[1/] are in response to the April 15, 1985 Federal Register notice inviting comments on "The Environmental Assessment and Finding of No Significant Impact" concerning the application to the National Institutes of Health (NIH), under the NIH Guidelines for Research Involving Recombinant DNA Molecules to Field Test Ice Nucleation Minus Bacteria to Control Frost Damage to Plants. Also included are comments on the need for a programmatic Environmental Impact Statement.

The Environmental Assessment and Finding of No Significant Impact

The NIH assessment appears to be well researched and documented. It represents a balanced, thorough and plausible evaluation of the environmental effects and impact of conducting research as proposed in the ice-nucleation-minus-bacteria project. It is apparent that all possible and relevant avenues of projected and potential concern were investigated and addressed. The document clearly goes beyond reasonable requirements to reassure the public that any possible field escape of the bacteria will have

[1/] The National Agricultural Chemicals Association (NACA) is a nonprofit, trade organization of manufacturers and formulators of pest control products employed in agricultural production. NACA's membership is composed of the companies which product and sell virtually all of the technical crop protection materials (active ingredients) and a large percentage of the formulated products registered for use in the United States.

no adverse effects on or in the environment. The expert consultants who were involved are world-renowned for their scientific credibility and judgment. As such, the Environmental Assessment clearly supports the continuation of the research and the conclusion that this experiment will not adversely affect the environment in any substantive manner.

The Need for a Programmatic Environmental Impact Statement

From our point of view, the Environmental Protection Agency and the U.S. Department of Agriculture, including APHIS, are taking leadership roles in agriculturally oriented research dealing with recombinant DNA and genetically engineered plant varieties. With the already published EPA interim guidelines dealing with pesticide research and notification, and the soon-to-be proposed rulemaking on the subject, it appears that the role of NIH, as oversight, should in the future be directed to only those activities of broad national concern involving public health issues. The technology of bio/genetic engineering, particularly in agriculture, is progressing in a very positive manner. The need for detached oversight such as the development of a very comprehensive Environmental Impact Statement by NIH to comply with certain interpreted requirements of NEPA seems now to be procedurally redundant unless there is a scientific reason to question a basically unknown revelation.

At this time, there is no reason to believe that genetically engineered organisms are any different than organisms that mutate during natural selection. Over the years, plant breeding techniques have used chemically induced mutations to assist in mass selection. In genetic engineering, the biological derivation is actually more precise and predictable. It would appear that very strong arguments would have to be made to justify the need for a programmatic Environmental Impact Statement to demonstrate that a genetically engineered organism has a greater chance to invoke environmental harm than an organism altered by traditional practice.

Very sincerely yours,

Earl C. Spurrier

cc: NACA ad hoc Committee on Biotechnology

THE UNIVERSITY OF NORTH CAROLINA
AT
CHAPEL HILL

School of Medicine
Department of Biochemistry & Nutrition

The University of North Carolina at Chapel Hill
Faculty Laboratory Office Building 231H
Chapel Hill, NC 27514-7231

May 16, 1985

Dr. William J. Gartland
ORDA
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20205

Dear Dr. Gartland:

I write as the President of the Association of Medical School Departments of Biochemistry to state that as the principal elected representative of my society, I believe I can give their general support for the Lindow and Panopoulous field test of ice-nucleation-minus bacteria prepared by recombinant DNA techniques.

I have read the NIH Environmental Assessment, the Federal Register, Vol. 50, No. 72, p. 455-456 (1985) and the text of a letter from the American Society of Microbiology Public and Scientific Affairs Board in response to the Federal Register Notice.

I believe the most crucial issue is that natural deletion mutants occur so that the recombinant deletion mutants cannot be considered unique. It is the process that produced the recombinant mutants that is unique. However, in addition, I believe the Environmental Assessment was exhaustive.

It is hard to alert all of the members of my Association which represents about 130 medical school biochemistry departments, but I am sure my view would represent a majority of the members if they had the information I do.

Sincerely,

Mary Ellen Jones

Mary Ellen Jones, Ph.D.
Kenan Professor and Chair

MEJ:lmh

ADVANCED GENETIC SCIENCES

6701 San Pablo Avenue, Oakland, CA 94608 (415) 547-2395

May 17, 1985

Dr. William J. Gartland
Director
Office of Recombinant DNA Activities
National Institute of Health
Building 31
Bethesda, Maryland 20205

Dear Dr. Gartland:

I have only a few comments on the Environmental Assessment (EA) prepared for the field experiment proposed by Drs. Lindow and Panopoulos and on the more general need for programmatic Environmental Impact Statement for field experiments with genetically engineered microbes. For having received both of these items from you, I am very grateful.

In my opinion the EA is a well prepared document that involves the expertise of some excellent research plant pathologists and well qualified advisors. I believe I also understand the basis for the requirement to complete such a document and Finding of No Significant Impact to satisfy legal requirements set for NIH. However, in reading the EA I was dismayed to find that such a large effort was expended to compile information that in my mind gives no greater assurance of safety than was available in the proposal of Lindow-Panopoulos that was approved by RAC. Except for site-specific information for Tule Lake, no substantially new information is brought forward to alleviate fears by documentation of experimental results. As no actual data is included or cited for many of the broad statements as to microbial competition, population dynamics, pathogenicity, etc., the statements as to risk or safety have diminished credibility.

My point is simply that this has been an unnecessary task asked of NIH and that the consideration and approval of the proposal by RAC, as submitted, was properly conducted with the currently available information in this subject area. The environmental and health risks were properly evaluated with the finding of no foreseeable impact from this limited field experiment. Much energy and effort was expended to restate the consensus of the NIH-RAC decision.

My only specific comment on the EA is that I disagree strongly with the use of Pseudomonas syringae pv syringae for epiphytic bacteria of this species which have no known pathogenicity to standard host plants. Until a host is found these strains could

be referred to as P. syringae thus avoiding the confusion as to their pathogenic associations.

The need for a programmatic EIS to address any release of genetically engineered organism is unlikely to be resolved in the near future. Environmental concerns and risks must be expressed on a case-by-case basis (as was the original approach of NIH-RAC) due to the large degree of variability in organism, target site, and introduced or altered gene function. I don't believe anyone can predict the diversity of potential basic or applied research needs that would cover all situations or sources of risk.

I do believe programmatic statements are sorely needed to define the expected environmental fitness of broad groupings of enginnered organisms and the potential risk of detectable presistance. It would be against this series of broad statements compiling currently available information that lay people and cross-disciplinary scientists could make preliminary judgements concerning risk to the environment. For example, where the genral need or intent of a research program is to release and test a biological control agent, the organism must be environmentally competent and competitive. This would be covered by different broad criteria than an engineered Rhizobium which can be introduced to plants in a way that avoids its competitive disadvantage to other native Rhizobium spp and can thus be tested, at a preliminary stage, without concern for its ability to persist. Similar examples can be drawn for differences between deletion mutations and novel expression or novel regulation of genes in the released organism, etc. Thus by compiling data to cover broad groupings of potential releases by, perhaps, experimental objective as it relates to environmental fitness and subdivided by function (i.e. toxin, antibiotic, enzyme, polysaccharide, etc.) guidelines covering experimental requirements prior to release might be more readily determined. Hopefully this could expedite consideration and review of release proposals by highlighting potential areas of concern for the researcher. More detailed analysis of the individual experiment could then refer to a more generalized environmental statement if the review committees find no significant risk in the proposed release. No additional comprehensive or site-specific EIS would be needed for small scale testing.

I hope this somewhat lengthy, and rambling letter will be helpful to your office in some way.

Sincerely,

Trevor Suslow

Trevor Suslow, Ph.D.
Director of Product Research
Staff Scientist Plant Pathology/Biocontrol

COMMENTS ON THE PUBLIC NOTICE 50 FED. REG. 14794 (1985) AND THE ENVIRONMENTAL ASSESSMENT, ETC.

NIH Office of Recombinant DNA Activities (ORDA)
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20205

These comments are submitted on behalf of the Foundation on Economic Trends, Inc., and the other plaintiffs in Foundation on Economic Trends v. Heckler, No. 83-2714, U.S. District Court, District of Columbia, in response to the public notice issued by the National Institutes of Health ("NIH"), 50 Fed. Reg. 14794 (April 15, 1985), and on the environmental assessment ("EA") and on the finding of no significant impact ("FONSI") referred to in the notice.

As a preliminary matter, we wish to point out a significant omission in the notice in its description of the opinion of the Court of Appeals in FET v. Heckler. The court of appeals agreed with the district court that the National Environmental Policy Act (NEPA) applies to the deliberate release experiments, the court stating "that if NIH fails to give appropriate environmental consideration to any other experiment, as it has failed to do with the University of California experiment, injunctive relief would be clearly proper." Slip op. at 4; see also Slip op. at 31.

The difficulty with the notice on this point is that it is consistent with -- and therefore could be erroneously construed to mean -- that the court held only that NEPA applies to the University of California

experiment. In light of the importance of the applicability of NEPA to all deliberate release experiments conducted by entities receiving recombinant DNA funding from NIH, a corrective public notice should be issued on this point.

Turning to the question of whether a programmatic EIS is necessary for the NIH activities involved in the review and the decisions on such experiments, we wish to state the public notice discussion of that issue is totally inadequate. The notice fails to reflect an objective and even handed approach to the question. The court of appeals made it abundantly clear that the NIH was under an obligation to undertake a "reasoned consideration" of the factors relevant to resolving that issue. Slip op. at 32. "Since NIH has given no serious consideration to whether a programmatic EIS is justified, we cannot evaluate its claims that deliberate release experiments are neither so 'cumulative' or 'connected' that a programmatic EIS is required under the CEQ regulations, nor so 'similar' that a programmatic EIS may not be 'the best way to assess adequately' their environmental effects. 40 C.F.R. [Sec.] 1508.25."

The public notice reflects a NIH's continued refusal to give serious consideration to the issue. The discussion of that issue consists primarily of a cryptic reiteration of the arguments made in court

against the notion that the NIH is required to prepare such a programmatic EIS, even though most of those arguments would be equally applicable to containment experiments, the guildelines for which prompted NIH to prepare an EIS. For the reasons stated in our appellate brief, which arguments we incorporate by this reference, and as indicated by the court of appeals opinion on the question, there are a number of factors suggesting that a programmatic EIS is required.

The public will not be adequately informed of the issues involved on the questions on whether a programmatic EIS is necessary unless there is at least a reasonable good faith attempt to set forth both sides of the issue in a public notice on the question. Accordingly, we request NIH to vacate the notice and issue an adequate one.

The environmental assessment on the application for the University of California experiment is inadequate. It contains many of the same shortcomings that were part of the NIH's approach to the issue in court. Again there was not an adequately interdisciplinary review of the experiment. Insofar as we have been able to determine, the scientists listed as consultants have not made direct research contributions in the discipline of ecology of micro-organisms. Dr. Kelman, the one scientist whose expertise borders on this area, has made his contributions primarily in the area of physiology of

parasitism, especially as regards bacteria. Another one is a virologist, and the others are predominantly genetic engineers. The assessment would have been improved had qualified ecologists in the relevant specialities been included as consultants. Issues can only be recognized and dealt with adequately if there are qualified experts undertaking the evaluation.

There was inadequate consideration of the nature of the modified Pseudomonas syringae ("Ps.") as highly competitive specimens chosen for their ability to displace their wildlife counterparts. Little or no attention was given to the genetic stability of these mutant bacteria, a point that Dr. Lindow emphasized distinguishes them from mutants created by chemical or radiation means. The implications for disruption of the natural balance between ice-nucleation-plus (INP) and ice-nucleation-minus (INM) Ps. is therefore not adequately considered.

Most of the other inadequacies of the environmental assessment are set forth in the declarations of Drs. David Pimentel and Stephen Richard Gliessman, copies of which are appended hereto and incorporated by this reference with the same effect as if fully set forth herein.

The EA fails to justify the conclusion that the experiment will have no significant environmental impacts because it fails to consider adequately the

risks involved. Accordingly, we request that the NIH withdraw the EA and the FONSI, conduct the additional experiments requested in these comments and make a new evaluation before issuing another EA. In light of the unresolved environmental issues and potential impacts involved, it would be preferable for the NIH to prepare an EIS on the experiment at the outset rather than going through the interim step of preparing an EA. Had the NIH done so at the outset, the EIS could have been completed by this time and it will undoubtedly prove to be necessary.

Edward Lee Rogers
Suite T-200
1718 P Street, N.W.
Washington, D.C. 20036
(202) 387-1600

May 14, 1985

Enclosures

DECLARATION OF DAVID PIMENTEL

David Pimentel declares:

1. I am David Pimentel. I reside at 147 North Sunset Drive, Ithaca, NY.

2. I am a terrestrial ecologist. My curriculum vitae, appended hereto, is incorporated by this reference with the same effect as though fully stated herein.

3. I have reviewed the environmental assessment and finding of no significant impact by the NIH proposed experiment by Drs. Steven Lindow and Nickolas Panopoulos of the University of California, at Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damaged plants. I have the following opinions and conclusions to make regarding that assessment and finding.

4. A statement is made that "neither the original nor the altered bacteria are pathogenic to living creatures" yet in Lindow's published papers he refers to this bacterium Pseudomonas syringae (Ps) as an "important pathogen" in nature. Lindow also speculates that Ps may well be pathogenic to insects. Thus, the statement in the assessment is incorrect.

5. It is stated that Ps "did not induce frost or other injury to plants." By plants is meant more than potato plants. Only a few tests were run with 6 crop species but no other crop plants that are cultured in that California region are included. Also no natural vegetation types were tested. Thus, insufficient data exist to support the quoted statement.

6. It is stated that "certain strains or isolates of Ps have been reported to be pathogenic on many woody and herbaceous plants including bean, lima bean, wheat, barley, sorghum, corn, pear, apple, plum, peach, cherry, citrus, and others." This statement contradicts an earlier quoted statement (see #4). Also, Lindow has not tested his Ps strain for its pathogenicity to all of these crop plants and again no natural plant species have been included in his environmental study.

7. It is stated that their strain of Ps "will have been screened for pathogenicity towards oats, wheat, barley, corn, tomato, and three cultivars of beans." When will these tests be completed and how will the scientific credibility of the data be validated? It should be noted that this list of crop plants makes up only a small portion of the total crop plants grown in the test area. In particular no fruit (pear, peach, plum, cherry, etc.) are included in the test nor are any natural plant species included.

8. It is stated that " Analogous to plant frost survival, the presence of INA^+ bacteria would be detrimental to survival of frost-sensitive insects and possibly beneficial to frost-tolerant insect species." This also contradicts an earlier statement that Ps is not "pathogenic to living creatures" (see #4). Lindow has not run any tests with beneficial insects like honey bees. Bees provide pollination to over $20 billion worth of U.S. crops annually. Other beneficial insects play an important role in biological control of pests.

9. Lindow should run adequate environmental studies with other crop and natural plant species and insects in the laboratory before his unique Ps bacterium is applied to test plots in the field. Then sound environmental tests must be designed to check for potential ecological impacts on fruit and other crops, important natural plants, and beneficial insects. As Lindow states the Ps organism has potential as an "important pathogen"; thus ample evidence exists that careful environmental studies should be run before Lindow's Ps organism is released.

10. Any proposed field experiments must be designed to limit dispersal of the released Ps organism. At the same time the field experiments should detail how dispersal and movement of the released Ps organism in air, soil, water, and various organisms like bees, other insects, and birds, will be monitored.

I declare under the penalty of perjury that the foregoing is true and correct.

DAVID PIMENTEL

DATE: March 14, 1985
Ithaca, NY

DAVID PIMENTEL

General
1925 Born May 24th at Fresno, California
1932-43 Educated in Massachusetts
1943-45 Pilot Officer-U.S. Air Force

Education
1943 St. John's University, Collegeville, Minn. (one semester)
1946 Clark University, Worcester, Mass. (one semester)
1945-48 University of Massachusetts, Amherst (B.S. Degree)
1948-51 Cornell University, Ithaca, New York (Ph.D. Degree)
1954-55 University of Chicago, Ill. (Post-doctorate, winters)
1961 Oxford University, England (O.E.E.C. Fellow)
1961 Massachusetts Institute of Technology, Cambridge, Mass. (NSF Computer Scholar during summer)

Positions
1948-51 Research Assistant, Department of Entomology and Limnology, Cornell University
1951-54 Chief, Tropical Research Laboratory, U.S. Public Health Service, San Juan, Puerto Rico
1954-55 Project Leader, Technical Development Laboratory, U.S. Public Health Service, Savannah, Georgia
1955-60 Assistant Professor of Insect Ecology, Department of Entomology and Limnology, Cornell University
1960-63 Associate Professor of Insect Ecology, Cornell University
1963-69 Professor and Head of Department of Entomology and Limnology, Cornell University
1973-74 Professor, Core Faculty, Center for Environmental Quality Management, Cornell University
1969-76 Professor of Insect Ecology, Department of Entomology and Section of Ecology and Systematics, Cornell University
1976- Professor of Insect Ecology & Agricultural Sciences, Department of Entomology and Section of Ecology and Systematics, Cornell University

Honors
Phi Kappa Phi, Sigma Xi, Organization for European Economic Cooperation Fellowship at Oxford University, NSF Computer Scholar. Invitational lectures at the following international congresses: XVI International Congress of Zoology (1963); XI International Congress of Genetics (1963); XII International Congress of Entomology (1964); Keynote Address Xth and XIth International Congress of Nutrition (1975, 1978).. Fellow of the American Association for the Advancement of Science. Fellow of the Canadian Entomological Society.

National Activities
1964-66 Panel on Environmental Pollution, President's Science Advisory Council
1966-68 Chairman, Panel on Biology and Renewable Resources, Committee on Life Sciences, National Academy of Sciences
1966-69 Management and Resources Committee, Int. Biol. Program
1967-68 Council, National Institute of Environmental Health Sciences
1967-70 Chairman, Training Committee, International Biological Program
1969 U.S. Delegate to UNESCO Conference on "University Governance and the Role of the Student"

National Activities (continued)
1969 Commission on Pesticides, Established by Secretary of Health, Education and Welfare

1969-70 Chairman, NSF Biological Panel (Advanced Training Projects)
1969-70 Ecologist Consultant on Environmental Quality, Executive Office of the President, Office of Science and Technology
1970 ad hoc Committee on Environmental Aspects of Foreign Assistance Programs, National Academy of Sciences
1970 Panel on "Water in Man's Life in India", National Academy of Sciences
1970- Editoral Advisory Board, Abstracts of Entomology, BioSciences Information Service of Biological Abstracts
1972-73 Co-chairman, Panel on Innovative Mosquito Control, Office of the Foreign Secretary, National Academy of Sciences
1972-73 Advisory Council on Environmental Education of the Office of Education
1972-73 Chairman, Panel on Environmental Impact of Herbicides, Environmental Protection Agency
1973-77 Chairman, Panel on Economic and Environmental Aspects of Pest Management in Central America, National Academy of Sciences
1974-76 Committee on Food and Food Production, National Academy of Sciences
1974-75 Committee on "World Food, Health, and Population", National Academy of Sciences
1974 World Food Conference, Consultant to the Food and Agriculture Organization of the United Nations
1975-79 Chairman, Board on Science and Technology for International Development, Office of the Foreign Secretary, National Academy of Sciences
1975-78 Chairman, National Advisory Council on Environmental Education, Office of Education, Department of Health, Education and Welfare
1975-78 Pesticide Advisory Council of the Environmental Protection Agency
1975-79 Commission on International Relations, National Academy of Sciences
1976-77 Chairman, Study Team on the Interdependencies of Food, Population, Health, Energy, and Environment, World Food and Nutrition Study, National Academy of Sciences
1977-78 Chairman, U.S. Advisory Committee to IIASA Program "Food and Agriculture", International Institute for Applied Systems Analysis, Austria
1979-81 Advisory Panel (Genetics), Office of Technology Assessment, U.S. Congress
1979-80 Chairman, Gasohol Panel, Energy Research Advisory Board, Department of Energy
1979-83 Chairman, Biomass Energy Panel, Energy Research Advisory Board, Department of Energy
1979-82 Chairman, Environmental Studies Board, National Academy of Sciences
1979-83 Member, USAID Research Advisory Committee, Department of State
1979- Member, AAAS Committee on Climate
1981- Vicechairman, Committee on Research Grants, Board on Science and Technology for Development, National Academy of Sciences
1980-82 Chairman, Land Productivity Panel, Office of Technology Assessment, U.S. Congress
1982-83 Member, Commission on Physical Sciences, Mathematics, and Resources, National Academy of Sciences
1982-83 Vicechairman, Solar R&D Panel, Energy Research Advisory Board, Department of Energy

1983- Chairman, Energy and Agriculture Panel, Energy Research Advisory Board, Department of Energy

Society Membership and Activities

Ecological Society of America
Entomological Society of America
Society for the Study of Evolution
Entomological Society of Canada
American Society of Zoologists
American Society of Naturalists
American Association for the Advancement of Science
American Institute of Biological Sciences
American Academy of Political and Social Science

Activities in the Entomological Society of America

1963 Chairman, Biological Control Section
1965 Chairman, Publicity and Public Relations Committee
1967 Chairman, National Medal of Science Committee
1968 Committee on Program Evaluation
1968-71 Governing Board
1971 Chairman, Editorial Board, Environmental Entomology
1973 Chairman, Screening Committee for Bussart and Geigy Awards
1974-75 President, Eastern Branch
1975-77 Chairman, Special Awards Committee
1977-80 Representative to American Association for the Advancement of Science

Activities in American Institute of Biological Sciences

1973- New York State Public Relations Legislative Representative

Activities in American Midland Naturalist

1973-77 Associate Editor

Activities in the Society for the Study of Evolution

1971-74 Councilor for the Society for the Study of Evolution

Activities in Agriculture, Ecosystems and Environment Journal

1979- Editorial Board

Activities in CRC Press

1981- Advisory Board for CRC Critical Reviews in Plant Sciences

Distinguished Lectures

Marine Sciences Distinguished Lecture Series, Louisiana State University, Baton Rouge, LA, 1979.

Gustavson Memorial Lecture, University of Colorado, Boulder, 1980.

The Fourth A.C. Neish Memorial Lecture, Nova Scotia Agricultural College, Truro, 1980.

Lectures on Science, Technology, and Society, Illinois Institute of Technology, Chicago, IL, 1981.

Declaration of Stephen Richard Gliessman

Stephen Richard Gliessman declares:

1. I am Stephen Richard Gliessman. I reside at Molino Creek Farm at the end of the Cement Plant Road Extension near Davenport, California with a mailing address of Post Office Box 69, Davenport, California, 95017.

2. I am an agroecologist. My curriculum vitae, appended hereto, is incorporated by this reference with the same effect as though fully stated herein.

3. I have reviewed the environmental assessment and finding of no significant impact by the NIH proposed experiment by Drs. Steven Lindow and Nickolas Panopoulos of the University of California, at Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damaged plants. I have the following opinions and conclusions to make regarding that assessment and finding.

4. It seems that all discussion of potential environmental hazards associated with the release of the altered bacteria depends on the minimization of risk and low probability of unexpected problems. The fact that this infers that potential problems are not wholly eliminated is reason for concern and greater caution in the possible release of the ice-nucleating negative organisms that have been altered with recombinant DNA techniques.

5. The assessment does not seem to deal with the question of what possible tests need to be designed or could be designed to ensure greater reduction or elimination of risk of adverse effects from release of the altered organism. Since this case could form a landmark decision for future tests of altered organisms, it is even more important that this first case serve as a model for the future.

6. Those consulted in the preparation of this assessment did not include an ecologist or evolutionary biologist. I feel that a broader range of expertise would be very important, especially since many of the aspects involved deal with ecological and evolutionary processes in the environment.

7. In the section of the assessment dealing with worst case considerations, the assumption again is made that potential problems are at a low probability, due to the presence already of ice nucleation negative bacteria in the environment. Before release takes place, it seems to me that it is necessary to gain further understanding of how a balance is maintained in nature between negative and positive bacteria. How do they shift? What factors promote one over the other? How can such information be used to ensure zero probability of problems once the altered bacteria are released? Have sufficient tests been performed to demonstrate that altered ice nucleation negative bacteria are exactly the same as naturally-occurring negative strains? It seems to me that a battery of tests of this type should be performed before release takes place.

I declare under penalty of perjury that the foregoing is true and correct.

Stephen Richard Gliessman

Date: March 11, 1985
Davenport, California

CURRICULUM VITAE

Dr. Stephen R. Gliessman

Address

Agroecology Program
Farm Project
Environmental Studies
University of California
Santa Cruz, California 95064

(408) 429-4051

Personal Information

Birthplace: San Francisco, California
Birthdate: 26 June 1946

Education (Undergraduate)

University of California, Santa Barbara, 1964-1968
Degree: B.A. in Botany
Major Courses of Study: Organic Chemistry, Plant Taxonomy, Plant Morphology, Community Ecology, Animal Ecology, Natural History, Anatomy of Plants, Plant Geography, Plant Physiology, Systemic Botany

Education (Graduate)

University of California, Santa Barbara, 1968-1972
Degrees: M.A. in Biology, 1970; PhD in Plant Ecology, 1972
Major Courses of Study: Advanced Systematics and Taxonomy, Plant/Soil Relations, Ecophysiology, Experimental Ecology, Plant Metabolism, Ecological Genetics, Tropical Biology

Doctoral Studies

Major: Plant Ecology
Minors: Plant Physiology and Plant Taxonomy
Advisors: Dr. C. H. Muller, Principal Advisor
Dr. M. F. Moseley
Dr. W. H. Muller
Dr. J. R. Haller
Dr. J. H. Connell
Thesis Title: The role of phytotoxins in the interference with associated plants by Bracken (Pteridium aquilinum) (L.) Kuhn

Languages

English (native), Spanish (fluent), German (reading)

Academic and Professional Experiences

1966-1967	Herbarium Assistant, UCSB Herbarium
1967-1968	Research Assistant, with Dr. C. H. Muller, UCSB
1968	Research Assistant, with Dr. R. H. Whittaker, UC Irvine
1968-1969	Teaching Assistant, Department of Biology, UCSB
1969	Participant, Course in Tropical Biology, OTS, Costa Rica
1969-1972	Recipient of an NDEA Graduate Fellowship
1971	Ecological Research in Mexico and Central America
1972	Professor, OTS Tropical Biology Course, Costa Rica
1972-1974	Resident Ecologist, Finca Loma Linda Experimental Farm, Costa Rica
1974-1976	General Manager, Jardines y Vivero Nursery, Guadalajara, Jalisco, Mexico
1976-1977	Professor/Investigator, Depto. de Ecologia, Colegio Superior de Agricultura Tropical, Cardenas, Tabasco, Mexico
1977-1980	Chairperson, Depto. de Ecologia, Colegio Superior de Agricultura Tropical, Cardenas, Tabasco, Mexico
1981-1984	Assistant Professor, Environmental Studies, University of California, Santa Cruz, California
1982-present	Director, Agroecology Program, University of California, Santa Cruz
1984-	Associate Professor, Environmental Studies, University of California, Santa Cruz

Professional Affiliations

Ecological Society of America
International Association of Ecology
Association for Tropical Biology
International Society of Tropical Ecology
British Ecological Society
Mexican Botanical Society
Internation Federation of Organic Agriculture
California Association of Family Farmers
Santa Cruz County Farm Bureau
American Association for the Advancement of Science

Major Publications

-Gliessman, S. R., 1980. Multiple cropping systems: a basis for developing an alternative agriculture. Prepared for the Office of Technological Assessment Workshop, Background Papers For Innovative Biological Technologies For Lesser Developed Countries, Washington, D.C., November, 1980.

-Gliessman, S. R., R. Garcia E., and M. Amador A., 1981. The ecological basis for the application of traditional agricultural technology in the management of tropical agro-ecosystems. Agro-Ecosystems 7:173-185.

-Gliessman, S. R., 1982. Nitrogen distribution in several traditional agroecosystems in the humid tropical lowlands of southeastern Mexico. Plant and Soil 67:105-117.

-Gliessman, S. R. and M. A. Altieri, 1982. Polyculture cropping has advantages. California Agriculture 36(7):14-16

-Gliessman, S. R., 1983. Allelopathic interactions in crop/weed mixtures: applications for weed management. J. Chemical Ecology 9:991-999.

-Gliessman, S. R., 1984. An agroecological approach to sustainable agriculture. In Jackson, W. & B. Colman (Eds.), To Meet the Expectations of the Land, Northpoint Press, Berkeley, California (in press).

National Institutes of Health

Recombinant DNA Research; Availability of Analysis of Public Comments

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice.

SUMMARY: A previous Federal Register notice (50 FR 14794–14796, April 15, 1985) announced the availability for public comment of the Environmental Assessment and Finding of No Significant Impact (EA–FONSI) dated January 21, 1985, "concerning the application to the National Institutes of Health (NIH), under the NIH Guidelines for Research Involving Recombinant DNA Molecules, of Drs. Steven Lindow and Nickolas Panopoulos of the University of California, Berkeley, to field test ice-nucleation-minus bacteria prepared by recombinant DNA techniques for purposes of biological control of frost damage to plants." In addition, the April 15, 1985, notice requested "comments on the need for a programmatic Environmental Impact Statement in connection with NIH approvals of proposals to release into the environment organisms containing recombinant DNA molecules." In response to the April 15, 1985, notice, fifteen letters were received. These letters were analyzed in a June 19, 1985, memorandum from Dr. Bernard Talbot, Deputy Director, National Institute of Allergy and Infectious Diseases, to Dr. James Wyngaarden, Director, NIH. This notice announces the availability of that June 19, 1985, memorandum, which is described below.

ADDRESS: The June 19, 1985, memorandum is available upon a request to the NIH Office of Recombinant DNA Activities (ORDA), Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland 20205.

FOR FURTHER INFORMATION CONTACT: Dr. William J. Gartland, ORDA, Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION: The June 19, 1985, memorandum, mentioned above, analyzes the letters received using the following structure:

- Analysis of Comments on January 21, 1985, EA–FONSI
  - Comments Praising the EA–FONSI
  - Comments Criticizing the EA–FONSI
  - Additional Comments and Response to Comments on EA–FONSI
- Analysis of Comments on Need for Programmatic EIS
  - Comments Indicating a Programmatic EIS is Not Required
  - Comment Indicating the Need for a Programmatic EIS "Is Unlikely to Be Resolved in the Near Future"
  - Comment Indicating a Programmatic EIS is Required
  - Response to Comments on Requirement for Programmatic EIS
- Comments and Response to Comments on Adequacy of April 15, 1985, Federal Register Notice.

Based on the analysis of the comments, thirteen recommendations were made. On July 2, 1985, Dr. James Wyngaarden, Director, NIH, concurred with each of the thirteen recommendations. Among the recommendations are: Specific changes to be made in the January 21, 1985, EA–FONSI which provide further clarification of the safety of the proposed field test; a reaffirmation of the adequacy of the EA–FONSI; a reaffirmation of the Finding of No Significant Impact; and a finding that a programmatic Environmental Impact Statement is not needed at this time.

Three attachments to the June 19, 1985, memorandum are: a copy of the January 21, 1985, EA–FONSI; a copy of the April 15, 1985, Federal Register notice; and copies of the public comments received.

Dated: July 15, 1985.

James B. Wyngaarden, M.D.,

Director, National Institutes of Health.

[FR Doc. 85–17397 Filed 7–22–85; 8:45 am]

BILLING CODE 4140–01–M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE
WORKING GROUP ON TOXINS
MINUTES OF MEETING[1]

August 16, 1985

The Working Group on Toxins of the Recombinant DNA Advisory Committee was convened at 9:00 a.m. on August 16, 1985, at the National Institutes of Health, Building 31A, Conference Room 4, 9000 Rockville Pike, Bethesda, Maryland 20892. The meeting was open to the public. Dr. Susan Gottesman was the Chair. The following people were present for all or part of the meeting:

Working Group Members:

John Collier
Sam Formal
Michael Gill
Susan Gottesman
William Habig
Dennis Kopecko
Myron Levine
Elizabeth Milewski
(Executive Secretary)

A working group roster is attached (Attachment I).

Other National Institutes of Health Staff:

Carol Anonsen, OD
William Gartland, NIAID
Malcolm Martin, NIAID
Stanley Nagle, NIAID
Gilbert Ogonji, OD

Others:

Tim Beardsley, Nature Magazine
Bill Bishai, Harvard Medical School
Jessie Boyd, University Hospital at Boston University Medical Center
Jeff Christy, Blue Sheet, FDC Reports, Inc.
Jack Murphy, University Hospital at Boston University Medical Center
John Newland, Uniformed Services University of the Health Sciences
Alison O'Brien, Uniformed Services University of the Health Sciences
John Williams, Beth Israel Hospital

[1]The working group is advisory to the RAC, and its recommendations should not be considered as final or accepted.

Dr. Gottesman, Chair, called the meeting of the Recombinant DNA Advisory Committee (RAC) Working Group on Toxins to order at 9:00 a.m. on August 16, 1985.

Dr. Gottesman said the current National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules require specific RAC review and NIH and Institutional Biosafety Committee (IBC) approval of certain experiments before initiation. Among these experiments are those involving deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxic molecules lethal for vertebrates at an LD_{50} of less than 100 nanograms per kilogram body weight. A specific appendix referring to the cloning of toxins was constructed in 1980 by the Working Group on Toxins. That appendix, entitled "Containment Conditions for Cloning of Genes Coding for the Biosynthesis of Molecules Toxic for Vertebrates," specifies the containment to be used for the deliberate cloning of genes coding for the biosynthesis of molecules toxic for vertebrates. The appendix was based on a consideration of the pharmacological toxicity of the toxin and specifies that experiments involving cloning of the genes for toxins such as botulinum toxins, tetanus toxin, diphtheria toxin, and Shigella dysenteriae neurotoxin must be reviewed by the RAC and have NIH and IBC approval before initiation.

Dr. Gottesman said three proposals involving two of these toxins are on the working group agenda for the August 16, 1985, meeting: (1) a request for permission to clone Shiga-like toxin (SLT) structural genes from bacterial species classified in the families Enterobacteriaceae or Vibrionaceae according to Bergey's Manual of Systematic Bacteriology (Attachment II); (2) a proposal to remove from Biosafety Level 4 (BL4) containment K-12 host-vector systems expressing a hybrid gene encoding α-melanocyte stimulating hormone (α-MSH) and portions of diphtheria toxin (Attachment III); and (3) a request for permission to construct a hybrid molecule in which the gene coding for interleukin-2 (IL-2) is joined to a segment of the gene encoding the A subunit and portions of the B subunit of diphtheria toxin. The hybrid gene would be cloned in E. coli K-12 host-vector systems (Attachment II).

Request for Permission to Clone Shiga-like Toxin Genes

Dr. Gottesman said Dr. Alison O'Brien of the Uniformed Services University of the Health Sciences (USUHS) was requesting permission to clone SLT structural genes (defined either by nucleotide sequence homology with SLT gene probes from E. coli or by antigenic cross-reactivity of their gene products with purified E. coli SLT) from bacterial species classified in the families Enterobacteriaceae or Vibrionaceae according to Bergey's Manual of Systematic Bacteriology into E. coli K-12 under the conditions specified in Federal Register Volume 49, Number 179. Dr. O'Brien had previously sought and had obtained permission from the NIH to clone SLT from E. coli in E. coli K-12 host-vector systems. The language of that permission appeared in Federal Register 49, Number 179, and was incorporated into the NIH Guidelines as Appendix F-IV-H.

Dr. Gottesman said Appendix F-IV-H of the NIH Guidelines currently reads:

"The intact structural gene(s) of the Shiga-like toxin from E. coli may be cloned in E. coli K-12 under BL3 + EK1 containment conditions.

"E. coli host vector systems expressing the Shiga-like toxin gene may be moved from BL3 to BL2 containment conditions provided that: (1) the amount of toxin produced by the modified host-vector systems is no greater than that produced by the positive control strain 933 E. coli 0157:H7, grown and measured under optimal conditions; and (2) the cloning vehicle is to be an EK1 vector preferably belonging to the class of poorly mobilizable plasmids such as pBR322, pBR328, and pBR325.

"Nontoxinogenic fragments of the Shiga-like toxin structural gene(s) may be moved from BL3 + EK1 to BL2 + EK1 containment conditions or such nontoxinogenic fragments may be directly cloned in E. coli K-12 under BL2 + EK1 conditions provided that the E. coli host-vector systems containing the fragments do not contain overlapping fragments which together would encompass the Shiga-like toxin structural gene(s)."

Drs. Habig and Levine said they might have conflict of interest considerations in dealing with the proposal from Dr. O'Brien as they both have collaborative associations with her. Dr. Gottesman suggested they participate in the discussion and vote on motions. Dr. Milewski would note their votes and the potential conflict of interest in the minutes of the meeting.

Dr. O'Brien said she and her group had been investigating organisms for the presence of SLT. SLT is defined as being cytotoxic to cells and antigenically cross-reactive with purified E. coli SLT. SLT has been identified in members of the families Enterobacteriaceae and the Vibrionaceae, and she was now requesting permission to clone SLT genes from the Vibrionaceae and the Enterobacteriaceae in E. coli K-12. The major goal of this work is to develop a cholera toxin vaccine.

Dr. O'Brien suggested SLT may be a common property of these bacteria; in organisms such as Shigella dysenteriae 1 SLT may play a role in virulence when a large amount of the protein is produced and other virulence factors are present.

Dr. O'Brien said SLT has also been recently identified in one strain of Campylobacter and two strains of Aeromonas. The Aeromonas are classified as Vibrionaceae. The Campylobacter had at one time been classified by Bergey's Manual as Vibrionaceae but had recently been reclassified as an orphan species.

Dr. O'Brien pointed out that a number of Campylobacter characteristics are common to the Vibrionaceae. She asked whether she might receive permission to clone SLT genes from Campylobacter.

Dr. Milewski said the August 19, 1985, Federal Register announcement (50 FR 33462) published 30 days before the RAC meeting and describing the proposal stated that Dr. O'Brien requested permission to clone the SLT gene from members of the families Enterobacteriaceae and Vibrionaceae as classified in Bergey's Manual. She suggested the working group first vote on the proposal as it

appeared in the Federal Register. The group could subsequently offer a recommendation on experiments involving Campylobacter.

Dr. Levine pointed out that investigators in other parts of the world may perform the types of experiments requested by Dr. O'Brien under much less restrictive conditions. He thought U.S investigators are unfairly shackled by differences in rules on the cloning of toxin genes.

Dr. Gottesman said there are two approaches to dealing with differences in rules between countries: (1) the working group may suggest the NIH Guidelines be modified; and (2) the investigator may request more generic approvals.

Dr. Gottesman suggested the working group proceed by voting on those portions of the request which were published in the August 19, 1985, Federal Register and subsequently propose and vote on other actions the working group deems reasonable.

Dr. Gottesman asked whether the SLT structural gene is carried on a phage. Dr. O'Brien replied that high toxin production in bacteria usually appears to be associated with the presence of SLT converting phages such as 933J and H19A/J but not always. A low level of toxin production can consistently be detected in the absence of phage, and may be associated with a chromosomal gene. Dr. O'Brien hypothesized that the phage gene may be a variant of the chromosomal gene. She suggested other variants of the gene might be found in other bacterial species. She added that the Shiga toxin gene appears to be a chromosomal gene in Shigella dysenteriae 1 since the phage cannot be induced.

Dr. Gottesman asked what is known about the host range of the SLT converting phages. Dr. John Newland of USUHS said the phages can infect other E. coli strains; other bacterial families have not been studied to determine whether these phages will infect members of these families.

Dr. Gottesman asked how much toxin was produced when the SLT gene under its own regulation on a multicopy plasmid was introduced into the E. coli host-vector system. Dr. O'Brien replied that a seven fold increase in toxin production compared to the standard strain 933 E. coli 0157:H7 occurs when the SLT gene under its own regulation is introduced into the E. coli host-vector system.

Dr. O'Brien said the issue of increased SLT production by the E. coli host-vector system should be kept in perspective. Under optimal laboratory conditions, Shigella dysenteriae 1 strain 60R produces 10^8 cytotoxic toxin doses per milligram (mg) protein per cell lysate; the standard strain 933 E. coli 0157:H7 produces 10^7 cytotoxic doses (CDs) per mg protein under optimal conditions. The E. coli host-vector strain carrying the SLT gene on a multicopy plasmid produces 7×10^7 CDs per mg protein under optimal conditions. This level of toxin production is still less than that produced by the Shigella dysenteriae 1 strain. She added that Shigella flexneri produces approximately 10^4 CDs per mg protein under optimal conditions. The Vibrio cholerae vaccine strain produces 10^4 CDs per mg protein under optimal conditions.

Dr. Collier pointed out that at this time the specific activity of SLT toxin is not known. Dr. Gottesman said arguments on mechanism of entry into the animal and treatability of symptoms support a recommendation to RAC that this proposal be recommended to the NIH Director. Dr. Formal said E. coli host-vector systems offer an additional safety factor since experience has shown E. coli K-12 will not ordinarily colonize the bowel.

Dr. Collier moved that the working group approve of Dr. O'Brien's request as it appears in the August 19, 1985, Federal Register. Dr. Kopecko seconded the motion.

Dr. Gill said he would like the record to show the working group was taking this action because it judged that E. coli host-vector systems carrying the SLT gene are no more harmful than Shiga toxin expressing strains found in nature.

By a vote of six in favor, none opposed, and no abstentions, the working group recommended RAC approve Dr. O'Brien's request as it appeared in the August 19, 1985, Federal Register. (Drs. Levine and Habig voted in support of the motion.)

Dr. Newland asked whether 933 E. coli 0157:H7 would be the standard against which activity is measured regardless of what strain was used as the source of the SLT gene. Dr. Gill said the strain specified in Appendix F-IV-H of the NIH Guidelines would be the standard strain regardless of the source of the SLT gene.

Dr. O'Brien asked the working group to consider substituting in Appendix F-IV-H a shigella strain which produces more toxin than 933 E. coli 0157:H7 as the standard against which to measure toxin production. Shigella dysenteriae 1 strains produce approximately 10^8 CDs per mg protein in cell lysate; standard strain 933 E. coli 0157:H7 produces 10^7 CDs per mg protein under optimal conditions.

Dr. Collier said the question is whether there is any reason to believe an E. coli K-12 host-vector strain expressing SLT or Shiga toxin on a high copy number plasmid would be any more toxic than Shigella found in nature. Dr. Levine pointed out that E. coli K-12 does not colonize the gut and a K-12 host-vector system carrying the SLT gene would not be more pathogenic than Shigella dysenteriae. He thought 10^8 CDs per mg protein in cell lysates would be an appropriate upper limit for permitting a clone to be removed from BL3 containment.

Dr. Gill said an extra margin of safety is provided by the specification in Appendix F-IV-H of 10^7 CDs per mg protein in cell lysates as the upper limit for removal of SLT clones from BL3 containment. He would prefer strain 933 E. coli 0157:H7 which produces 10^7 CDs under optimal containment be the standard strain for determining whether a particular host-vector strain could be removed from BL3 containment. If cloning of the Shiga toxin gene on a high copy number plasmid in E. coli K-12 resulted in a 10 fold increase in toxin production, a very high level of toxin, 10^9 CDs, would be produced.

Dr. Kopecko said it was important to clone the Shiga gene from Shigella dysenteriae 1 in order to understand how toxin gene expression is controlled since Shigella dysenteriae 1, an epidemic strain, produces more toxin than other bacteria. He thought 10^8 CDs per mg protein is equivalent to the highest level of Shiga toxin expression found in nature; therefore, the working group should recommend E. coli K-12 host-vector systems expressing 10^8 CDs per mg protein be permitted to be moved from BL3 to lower containment. K-12 strains producing more than 10^8 CDs per mg protein will not be permitted to leave BL3 containment under the proposed language.

Dr. Gottesman said the issue is whether E. coli K-12 producing high levels of Shiga toxin would be hazardous to animals.

Dr. Habig said he did not see why E. coli K-12 host-vector systems carrying the Shiga toxin gene would pose a greater danger than Shigella dysenteriae 1. Dr. Kopecko pointed out that clinical laboratories routinely work with Shigella dysenteriae 1 at BL2 conditions. He did not think E. coli K-12 host-vector systems would be more dangerous than Shigella dysenteriae 1. Dr. Gottesman said she felt comfortable with permitting E. coli host-vector systems expressing 10^8 CDs of SLT to be used at BL2 containment since E. coli K-12 does not colonize the gut.

Dr. Levine suggested a number such as 10^8 CDs be chosen as the upper limit of toxin a host-vector system might express if it is to be removed from BL3 to a lower containment level.

Dr. Levine said E. coli K-12 is not an invasive pathogenic organism. Several factors are necessary for pathogenicity, and even for Shigella more is required than simply expression of the toxin gene. An attenuated Shiga bacillus vaccine strain which produces as much Shiga toxin as the parent pathogenic strain but lacks invasiveness factors did not cause disease when fed to human volunteers. In a the single case in which the strain reverted to invasive, the human volunteer became ill.

Dr. Formal said he had constructed a strain by introducing half of the Shigella flexneri chromosome and an SLT converting phage into E. coli K-12. The introduced Shigella flexneri genes were thought to include all of the genes necessary for virulence. The constructed strain had no effect on monkeys in feeding experiments although it produced 10^7 CDs per mg protein of SLT under optimal laboratory conditions.

Dr. Gill asked whether concern exists about transfer to other organisms of a high copy number plasmid carrying the Shiga toxin gene and its control elements.

Dr. Newland said the probability of transfer of the SLT or Shiga toxin gene by phage in nature is higher than the probability of transfer from K-12 by poorly mobilizable plasmid vectors.

Dr. Gottesman asked what laboratory conditions are necessary to obtain optimal expression of the SLT gene in the E. coli K-12 host-vector system. Dr. Newland replied that antibiotic pressure is necessary. In addition, several other procedures such as using specially treated medium are also required. If an

SLT clone is simply cultured in the conditions usually used to culture the host-vector system, toxin production is generally a factor of 10^2 lower than the highest level of expression obtained under optimal conditions with that clone.

Dr. Newland said he had observed that the recombinant plasmid carrying the SLT gene appears to be rapidly lost from the E. coli host-vector system in the absence of selective antibiotic pressure. The plasmid without the SLT gene is not so rapidly lost in the absence of selective pressure. He and Dr. O'Brien hypothesized that high levels of toxin may be toxic to bacteria. The rapid loss of the recombinant plasmid in the absence of selective pressure may be an additional safety factor.

Dr. Gottesman asked whether the working group would consider modifying Appendix F-IV-H by raising the upper level of toxin expression to 10^8 CDs per mg protein in cell lysates in exchange for an assurance that the plasmid will be rapidly lost in the absence of antibiotic pressure. Alternatively, an upper level of expression need not be fixed if the plasmid will be rapidly lost since rapid plasmid loss will provide a measure of safety.

Dr. Levine said one problem with Dr. Gottesman's proposal is that language generic for experiments involving E. coli may not apply to experiments involving Vibrio or Shigella. A second concern is the variability of the SLT assay; without an internal standard it is difficult to know how much toxin is produced. He asked Dr. O'Brien how great a difference is observed using the same procedure from experiment to experiment. Dr. O'Brien replied that a 100 fold difference in the amount of CDs produced may be observed from experiment to experiment.

Dr. Gill moved that Appendix F-IV-H be amended to read in part:

> "E. coli host-vector systems expressing the Shiga-like toxin gene product may be moved from BL3 + EK1 to BL2 + EK1 containment conditions provided that: (1) the amount of toxin produced by the modified host vector systems be no greater than that produced by the positive control strain 933 E. coli 0157:H7, grown and measured under optimal conditions, or ten times this level if the maintenance of the plasmid carrying the gene is dependent upon growth in the presence of an antibiotic...."

Dr. Levine suggested the motion be amended to read in part:

> "E. coli host-vector systems expressing the Shiga-like toxin gene product may be moved from BL3 + EK1 to BL2 + EK1 containment conditions provided that: (1) the amount of toxin produced by the modified host vector strain be no greater than 10^8 CDs per mg protein...."

Dr. Formal suggested the motion should name a toxin producing strain as the standard rather than citing a specific number. He suggested Shigella dysenteriae 1 strain 60R be used for this purpose. Shigella dysenteriae 1 strain 60R, a rough strain not capable of colonizing the human gut, produces about ten

times more toxin than 933 E. coli 0157:H7. Language specifying a specific strain would provide an internal standard and permit comparison from laboratory to laboratory. Dr. Kopecko agreed.

Dr. Levine amended his motion to require use of Shigella dysenteriae strain 60R as the standard strain. Dr. Formal seconded this motion.

Dr. Gottesman seconded Dr. Gill's original motion in order to permit a vote on this motion. By a vote of two in favor, four opposed, and no abstentions, Dr. Gill's motion was refused by the working group. (Dr. Levine opposed this motion. Dr. Habig supported it.)

The working group then voted on Dr. Levine's motion. By a vote of five in favor, one opposed, and no abstentions, the working group accepted this motion. (Drs. Habig and Levine supported this motion.)

Dr. Kopecko then offered the following motion:

> "Campylobacter species have long been recognized as members of the family Vibrionaceae. Recently Campylobacter species have been separated taxonomically into an orphan genus. Because of the similarities between Campylobacter and Vibrionaceae, the Working Group on Toxins recommends that for purposes of cloning SLT Campylobacter can be considered as members of the Vibrionaceae."

Dr. Levine seconded this motion.

Dr. Gill said the most important consideration in evaluating a proposal is not the species from which the gene was originally isolated but the characteristics of the gene's product and the host-vector system. He said he would support Dr. O'Brien's request to permit cloning of the SLT gene from Campylobacter. He thought Dr. Kopecko's motion did not convey the idea that the gene, and the characteristics of its product are the most important considerations. He suggested a substitute motion which would not refer to the source of the gene be developed.

Dr. Formal said although he agreed with Dr. Gill, at the present time it would be difficult to precisely define the characteristics of SLT toxin. A substitute motion would have to contain such a definition.

Dr. Milewski said Dr. Kopecko's motion was preferable because of the way the August 19, 1985, Federal Register annoucement had been written. She said the minutes of the working group meeting would show the group strongly supports the concept that the gene is the most important consideration.

Dr. Levine called the vote on Dr. Kopecko's motion. By a vote of six in favor, none opposed, and no abstentions, the working group approved of the motion. (Drs. Levine and Habig supported the motion.)

Request to Remove Clones Carrying the α-MSH-Diphtheria Toxin Gene From High Containment

Dr. Gottesman said Dr. John Murphy, then of Harvard Medical School and now with the University Hospital of the Boston University Medical Center, in a letter dated October 5, 1982, requested permission to construct a hybrid molecule in which the gene coding for α-melanocyte stimulating hormone (α-MSH) is joined to a segment of the gene encoding diphtheria toxin. The gene segment would encode the A subunit and portions of the B subunit of the diphtheria toxin gene. The segment would not contain the diphtheria toxin binding domain. The α-MSH gene would be a synthetic oligonucleotide. The α-MSH-diphtheria toxin hybrid gene would be introduced into poorly mobilizable plasmids such as pBR322, PUC9, or PUC8 and cloned in E. coli EK1 host-vector systems. The goal of these experiments is to construct a family of chimeric toxin genes whose products have the potency of diphtheria toxin and the receptor binding specificity of α-MSH.

This request was discussed by the RAC at the October 25, 1982, meeting; RAC recommended these experiments be permitted at BL4 containment at the Frederick Cancer Research Facility. The RAC also recommended that data on the characteristics of the recombinant organisms expressing the α-MSH-diphtheria toxin hybrid gene should be evaluated before the clones are permitted to leave the BL4 facility. The RAC charged the Working Group on Toxins with reviewing the data on the E. coli strains carrying the hybrid toxin; the group also was charged with offering a recommendation to the Director, NIH, on whether the strains may be removed from BL4 containment. The recommendation of the Working Group on Toxins could be acted upon by the NIH Director without RAC review of the data. A report of the Working Group on Toxins would, however, be sent to the RAC.

Dr. Gottesman said Dr. Murphy made the following three separate requests concerning removal from the BL4 facility of the E. coli K-12 host-vector systems:

(1) that fragments of the diphtheria toxin structural gene (expressed from either the tox or lambda phage P_R promotor) which include fragment A and fragment B up to, but not beyond, the SphI site be classified at BL1;

(2) that the diphtheria toxin structural gene modified by the introduction of a C-terminal cysteine residue up to, but not beyond, the SphI site (pARC508) be classified at BL1;

(3) that the diphtheria toxin-related α-MSH fusion genes up to, but not beyond, the SphI (pABM508, or pABM1508) site be classified at BL1.

Dr. Gottesman said Dr. Murphy had supplied data in the form of two tables. Table 1 contains data on intra-peritoneal injection challenge of guinea pigs with viable and boiled Escherichia coli host-vector systems and E. coli pABM1508 carrying the α-MSH diphtheria toxin fusion gene. Table 2 contains data on guinea pig survival after injection with partially purified conjugate protein at graded doses up to 1000 minimal lethal dose equivalents (based on 160 nanograms diphtheria toxin per kg body weight as minimal lethal dose.)

Dr. Murphy said these data suggest E. coli host-vector systems carrying the hybrid gene are not harmful to guinea pigs. Table 2 data suggest that up to 1000 equivalent diphtheria toxin doses of conjugate protein challenges to guinea pigs cause no effect. He said one animal died during the course of these experiments; autopsy showed no evidence of the pathology of the adrenal glands characteristic of diphtheria toxin intoxication.

Dr. Gill said the crucial question in review is whether lack of human toxicity can be predicted from the data submitted by Dr. Murphy. He said one question is whether the α-MSH-diphtheria toxin hybrid protein could be lethal to humans or whether an exposed individual might become albino. He said it is not known whether destroying cells possessing α-MSH receptors would be lethal to a human.

Dr. Habig asked whether human α-MSH would be recognized by guinea pig α-MSH receptors. Dr. Murphy said human α-MSH is recognized by guinea pig melanocytes.

Dr. Gottesman asked Dr. Murphy what cells having α-MSH receptors would have been encountered by the toxin during the guinea pig tests. Dr. Murphy said skin melanocytes in the G2 phase of the division cycle are sensitive to diphtheria toxin. Cells in the brain also have MSH receptors but would not be exposed in these tests because of the blood-brain barrier.

Dr. Kopecko asked whether the guinea pig has α-MSH receptor-possessing cells in the peritoneum. Dr. Murphy said guinea pigs are very sensitive to intraperitoneally introduced diphtheria toxin.

Dr. Gill asked Dr. Murphy if he knew whether guinea pig melanocytes were destroyed during the test. Dr. Murphy said he did not know if melanocytes had been destroyed.

Dr. Levine asked how long the animals had been observed during these tests. Dr. Murphy said the animals had been observed for at least 150 hours. This is approximately 24 hours beyond the observation period employed when animals are challenged with one minimal lethal dose equivalent of diphtheria toxin.

Dr. Murphy said diphtheria toxin is only fatal to sensitive cells in the G2 portion of the cycle since sensitive cells only express α-MSH receptor in the G2 phase of division. Normal melanocytes have a very low basal rate of protein synthesis and replicate about once a year. Therefore, only a very small fraction of the melanocyte population would be expressing the α-MSH receptor at a given time. In order to envisage fatality, either a long colonization period by toxin producing bacteria or a long serum half life of the α-MSH-diphtheria toxin hybrid molecule would have to be hypothesized.

Dr. Habig asked whether the α-MSH-diphtheria toxin hybrid protein could enter brain cells through hydrophobic insertion if administered intracerebrally or intraneurally. Dr. Murphy said the conjugate toxin probably is as potent as the CRM 45 fragment when injected intracerebrally or intraneurally.

Dr. Habig said diphtheria toxoids with an intact A chain possess a fair amount of residual enzymatic activity. He asked Dr. Murphy if he had any data on the

toxicity of the Sphl fragment. Is the Sphl fragment an active toxin if the α-MSH moiety is cleaved off? Is the level of toxicity of the Sphl fragment similar to that of the CRM 45 fragment? He noted that the CRM 45 fragment is smaller than Sphl but is toxic injected intracerebrally or intraneurally.

Dr. Gill pointed out that the CRM 45 fragment is 100 times less toxic than diphtheria-toxin. If the Sphl fragment is 100 times less toxic than diphtheria toxin, experiments involving the cloning of the fragment would be classified in the BL1 category.

Dr. Collier asked Dr. Murphy to compare the Sphl fragment to the CRM fragments in terms of size. Dr. Murphy said the Sphl fragment lacks 67 amino acids of the mature diphtheria toxin. It is larger than the largest known CRM fragment.

Dr. Gill asked if any data exist on the toxicity of a diphtheria toxin fragment of that size. Dr. Murphy said no data exist but felt the Sphl fragment would be as toxic as the CRM 45 fragment.

Dr. Levine asked whether the challenged guinea pigs mounted an antibody response against the α-MSH-diphtheria toxin hybrid protein. Dr. Murphy said he had not tested for an antibody response but assumed antibodies would be produced.

Dr. Habig asked whether the conjugate protein could cause an antibody response against α-MSH itself. Dr. Levine said another question is whether the conjugate would be effective in humans if more than one course of therapy was necessary since most humans would mount an antibody response against the α-MSH-diphtheria toxin hybrid molecule.

Dr. Habig said 80 per cent of the immunized population have some antibodies to the A chain portion of the diphtheria molecule. He questioned whether the α-MSH-diphtheria toxin molecule would be therapeutically effective in immunized populations. Dr. Murphy hypothesized that it would. He said only those monoclonal antibodies that block toxin binding to the receptor neutralize the toxin; monoclonal antibodies that bind to the toxin but do not block receptor binding do not neutralize the toxin. He hoped that only antitoxin antibodies capable of blocking receptor binding would be able to neutralize the α-MSH-diphtheria toxin molecule *in vivo*.

Dr. Habig said the α-MSH-diphtheria toxin hybrid might be neutralized in the animal by the formation of polyvalent complexes. Tetanus toxin can be neutralized by lattice formation even if the receptor binding site is not blocked by antibodies.

Dr. Gottesman asked whether fragment Sphl has an internal stop codon. Would fusion proteins result if "read-through" occurred? Dr. Murphy said the Sphl fragment has no internal stop codon. A fusion protein results when the Sphl gene fragment is linked directly to the vector DNA.

Dr. Gottesman asked Dr. Murphy whether any toxicity data exist for random Sphl fusion molecules. Dr. Murphy said he had no toxicity data on Sphl fusion

proteins. He felt the fusion proteins would be unstable and rapidly degraded in the bacteria and in crude lysate preparations.

Dr. Gill said this later possibility could be tested by spiking the preparation with whole purified diphtheria toxin. This would give a limited answer, however, since fusion proteins may be degraded at different rates than the whole toxin.

Dr. Habig said some toxicity questions could be answered by permitting the α-MSH-diphtheria toxin hybrid protein to be purified and tested in clinical trials.

Dr. Gottesman said the working group must deal with two questions. The first is whether fusing the SphI fragment with the α-MSH gene would confer on the hybrid molecule properties quite different from the properties of diphtheria toxin. The second is the toxicity of the SphI fragment and any randomly generated fusion proteins.

Dr. Formal said the crux of the matter is whether the host-vector system carrying the hybrid molecule is safe. One important question is whether diphtheria toxin could be produced by E. coli K-12 in the lumen of the bowel. Dr. Formal said there is no evidence K-12 produces diphtheria toxin in the bowel.

Dr. Malcolm Martin of the National Institute of Allergy and Infectious Diseases (NIAID) said the working group should focus on an evaluation of the E. coli host-vector system carrying the hybrid gene rather than on the toxicity of the hybrid toxin.

Dr. Gottesman said Appendix F of the NIH Guidelines is based on the pharmacological activity of toxins. The working group, therefore, would consider toxin toxicity. Dr. Gill agreed.

Dr. Gottesman said if the α-MSH-SphI conjugate protein is not as toxic as diphtheria toxin, then containment lower than BL4 is indicated. She asked whether the working group is willing to lower containment to the BL1 level for procedures involving the α-MSH-SphI conjugate protein.

Dr. Collier said a judgment call was made when diphtheria toxin was classified in Appendix F of the NIH Guidelines in 1980. On the basis of diphtheria toxin toxicity, this toxin might have been classified as requiring either BL3 or BL4 containment. He thought the BL4 containment assigned at that time was inappropriately high. He pointed out that Appendix F permits the cloning at BL1 + EK1 of Pseudomonas aeruginosa exotoxin A which is only ten to fifty times less toxic than diphtheria toxin.

Dr. Habig said the pathogen elaborating diphtheria toxin, Corynebacterium diphtheriae, is grown at BL2 containment in clinical laboratories and large culture production. The NIH Guidelines also require BL2 containment for work with Corynebacterium diphtheriae.

Dr. Kopecko said E. coli K-12 host-vector systems are not pathogens. He thought the worst case scenario would involve the potential transfer of the recombinant DNA plasmid to another organism. Dr. Levine said even if the E. coli K-12 host-vector system could colonize the gut the rate of transfer of plasmids in the gut is extremely low under normal conditions.

Dr. Murphy said all of the conjugate protein produced by the E. coli host-vector system is exported to the periplasmic compartment. Dr. Habig asked if this was the case even in the stationary phase of bacterial growth. Dr. Murphy said he did not know but reminded the group that experiments involving intra-peritoneal injection of viable and heat killed organisms showed no different response to organisms carrying the hybrid gene and the host-vector system without the hybrid gene.

Dr. Levine said he was not sure the observation the protein was not secreted is an argument for safety. Bacterial cells are not immortal and will lyse and secrete the fusion protein into the immediate environment.

Dr. Murphy thought a large number of cells would have to lyse to produce protein levels lethal to the animal.

Dr. Gill questioned whether the toxicity data presented by Dr. Murphy might have been generated using degraded conjugate protein.

Dr. Habig asked Dr. Murphy whether he had ascertained that the α-MSH-diphtheria toxin hybrid protein was isolated intact. Dr. Murphy said he had not looked at the C terminal sequences to determine whether the hybrid protein had been degraded. The active fraction was homogeneous on gels and was close to the deduced molecular weight of 50,000, however.

Dr. Collier asked Dr. Murphy how he knew the fraction injected into the guinea pigs contained active α-MSH-diphtheria toxin hybrid protein. Dr. Murphy replied that the protein fraction was active against sensitive cells in culture.

Dr. Gottesman asked how sensitive these tissue culture cells are to diphtheria toxin. Dr. Murphy replied that the control cells, African green monkey CV-1 cells, are sensitive to 10^{-10} or 10^{-11} molar diphtheria toxin.

Dr. Gill said the important considerations are the toxicity of the SphI fragments and of the conjugate protein. He thought the toxicity of the SphI fragment is very likely to be very low; however, it is not possible in BL4 conditions to obtain sufficient purified material to determine toxicity.

Dr. Gill suggested Dr. Murphy be given permission to work in BL3 conditions to purify enough material for toxicity testing and to quantitatively assay the hybrid protein in more species of animals over a longer period of time. Drs. Gottesman and Collier agreed that Dr. Murphy should be permitted to remove the clones from BL4 containment for the purpose of obtaining additional data.

Dr. Levine said he would prefer Dr. Murphy be permitted to use BL2 containment. He moved that BL2 be recommended as the containment level for Dr. Murphy's three requests, and that this language be recommended for approval by the working group. This motion would permit Dr. Murphy to produce enough material to test toxicity. Dr. Formal seconded this motion.

Dr. Collier re-emphasized that the toxicity of the SphI fragment is not known. He offered a friendly amendment to Dr. Levine's motion; the modified motion would specify BL3 containment conditions for Dr. Murphy's three proposals. Dr. Levine accepted this modification.

Dr. Martin suggested the working group specify BL2 containment plus BL3 practices for Dr. Murphy's proposals. A negatively pressurized room is the only difference between BL3 containment and BL2 containment plus BL3 procedures. He did not think a negatively pressurized room offered any additional safety factor for Dr. Murphy's proposed experiments.

Dr. Levine modified his motion to require BL2 containment plus BL3 practices for Dr. Murphy's three requests.

Dr. Gill suggested additional language be added to the motion to indicate the working group wished to permit Dr. Murphy the opportunity to acquire better toxicity data on the purified protein with the implication containment could be further lowered on the basis of these data.

Dr. Levine accepted Dr. Gill's proposed amendment.

By a vote of six in favor, none opposed, and no abstentions, the working group approved of Dr. Levine's motion.

Proposal to Construct a IL-2-Diphtheria Toxin Hybrid Protein

Dr. Gottesman said in this proposal, Dr. John Murphy of the University Hospital of the Boston University Medical Center requests permission to construct a hybrid gene composed of the gene coding for interleukin-2 (IL-2) and the SphI segment of the diphtheria toxin gene. The hybrid gene would be cloned in *E. coli* K-12 host-vector systems.

The long-term goal of this research is to develop novel IL-2 receptor-targeted cytotoxic agents to combat organ rejection and to create a state of graft "tolerance" in organ transplantation. Some of the products may have therapeutic potential for treating leukemia and lymphoma.

Dr. Gottesman said the primary difference between the α-MSH-diphtheria toxin hybrid molecule proposal and the IL-2-diphtheria toxin hybrid molecule proposal is the type of cell postulated to be sensitive to the conjugate protein.

Dr. John Williams of Beth Israel Hospital said IL-2 receptors are found on proliferating, antigen-activated T cells and to a lesser extent on activated B cells. These cells would be the targets of the IL-2-diphtheria toxin hybrid

protein. Resting or memory lymphocytes do not express IL-2 receptors. There is no evidence of IL-2 receptors on tissues outside of the lymphoid compartment.

Dr. Williams said the goal of this project is to selectively remove activated T cells and activated B cells during a well controlled time period following organ transplantation. It is hoped the IL-2-diphtheria toxin molecule would be more selective than the immunosuppressive techniques currently in use. Experiments in mice using anti-IL-2 receptor-monoclonal antibody conjugates to suppress cells which cause rejection in cardiac transplants support this hypothesis. In these experiments, the life of the graft in mice was extended from 10-14 days to three months or more in 80 percent of the animals.

Dr. Gill said the "worst case" scenario he could conjecture would be that an animal's T cells and B cells would be eliminated by this hybrid protein. A long observation of test animals might be necessary before such an effect would be detected. Dr. Habig said a long observation period might also be necessary for animals treated with the α-MSH-diphtheria hybrid protein.

Dr. Levine said the IL-2-diphtheria toxin hybrid protein could be an important therapeutic agent, but it would be a new toxin and could be harmful. He suggested containment be initially set at the BL4 level.

Dr. Martin said the largest BL4 facility at the Frederick Cancer Research Facility is run by NIAID. Any request by Dr. Murphy for use of the facility will need to be considered by NIAID in relation to other competing requests.

Dr. Levine said since little in known about the potential toxicity of the proposed hybrid protein, the working group approach should be conservative. If BL4 containment is not available, the experiment should be permitted under BL3 containment with a recognition that concern exists about the nature of the proposed hybrid protein.

Dr. Gottesman suggested containment could be set at BL3 with a requirement for the use of EK2 host-vector systems. Dr. Levine said he would accept the suggestion to specify EK2 vectors; he would not accept the suggestion to specify EK2 hosts.

Dr. Gottesman asked whether any type of risk assessment experiment could be performed which would answer basic questions about the nature of the protein.

Dr. Gill said risk assessment experiments with this hybrid protein would differ from previous risk assessment protocols because acute lethality is not a likely outcome. Tests would have to be devised to look for chronic impairment of the immune system. This type of data and the protocols necessary to generate it are different from that generally required for toxins.

Dr. Gill offered a motion to recommend permission to proceed under BL3 conditions; EK2 vectors would be used. Dr. Levine said he would prefer the language of the motion specified the use of "poorly mobilizable plasmid vectors such as the EK2 certified plasmids." Dr. Gill agreed to this modification. Dr. Levine seconded the motion.

By a vote of five in favor, none opposed, and no abstentions, the working group agreed this recommendation should be forwarded to the RAC.

The meeting of the Working Group on Toxins was adjourned at 12:20 p.m.

Respectfully submitted,

Elizabeth Milewski

Elizabeth A. Milewski, Ph.D.
Rapporteur and
Executive Secretary

9/23/85
Date

Susan Gottesman

Susan Gottesman, Ph.D.
Chair

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ATTACHMENT II OF THE MINUTES OF THE AUGUST 16, 1985, MEETING OF THE RAC WORKING GROUP ON TOXINS IS PAGE 33462 OF THE AUGUST 19, 1985, FEDERAL REGISTER WHICH CAN BE FOUND IN VOLUME 10 OF RECOMBINANT DNA RESEARCH.

Federal Register

Monday
August 19, 1985

Part IV

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Advisory Committee Meeting; Notice

Recombinant DNA Molecules Research, Proposed Actions Under Guidelines; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Advisory Committee; Meeting

Pursuant to Pub. L. 92–463, notice is hereby given of a meeting of the Recombinant DNA Advisory Committee at the National Institutes of Health, Building 31C, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20205, on September 23, 1985, from 9:00 a.m. to adjournment at approximately 5:00 p.m. This meeting will be open to the public to discuss:

Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols;
Containment for cloning of toxin genes;
Exchange list for gram positive bacteria;
Amendment of Guidelines; and
Other matters to be considered by the Committee.

Attendance by the public will be limited to space available. Members of the public wishing to speak at the meeting may be given such opportunity at the discretion of the chair.

Dr. William J. Gartland, Jr., Executive Secretary, Recombinant DNA Advisory Committee, National Institutes of Health, Building 31, Room 3B10, telephone (301) 496–6051, will provide materials to be discussed at the meeting, rosters of committee members, and substantive program information. A summary of the meeting will be available at a later date.

Dated: August 13, 1985.

Thomas E. Malone

Deputy Director, NIH.

(OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.)

[FR Doc. 85–19719 Filed 8–16–85; 8:45 am]

BILLING CODE 4140–01–M

Recombinant DNA Research; Proposed Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, DHHS.

ACTION: Notice of Proposed Actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth proposed actions to be taken under the NIH Guidelines for Research Involving Recombinant DNA Molecules. Interested parties are invited to submit comments concerning these proposals. After consideration of these proposals and comments by the NIH Recombinant DNA Advisory Committee (RAC) at its meeting on September 23, 1985, the Director of the National Institutes of Health will issue decisions on these proposals in accord with the Guidelines.

DATE: Comments must be received by September 18, 1985.

ADDRESS: Written comments and recommendations should be submitted to the Director, Office of Recombinant DNA Activities, Building 31, Room 3B10, National Institutes of Health, Bethesda, Maryland 20205. All comments received in timely response to this notice will be considered and will be available for public inspection in the above office on weekdays between the hours of 8:30 a.m. and 5:00 p.m. Comments received by close of business September 16, 1985, will be reproduced and distributed to the RAC for consideration at its September 23, 1985, meeting.

FOR FURTHER INFORMATION CONTACT: Background documentation and additional information can be obtained from Drs. Stanley Barban and Elizabeth Milewski, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205, (301) 496–6051.

SUPPLEMENTARY INFORMATION: The National Institutes of Health will consider the following actions under the Guidelines for Research Involving Recombinant DNA Molecules.

I. Request for Permission To Clone Shiga-like Toxin From the Families Enterobacteriaceae and Vibrionaceae in E. coli K–12

Dr. Alison O'Brien of the Uniformed Services University of the Health Sciences had previously (49 FR 36052) received permission from the National Institutes of Health to clone the structural gene of the Shiga-like toxin (SLT) of *Escherichia coli* in *E. coli* K–12. The specific language concerning the permission reads as follows in the current version of the NIH Guidelines (49 FR 46279):

> Appendix F–IV–H. The intact structural gene(s) of the Shiga-like toxin from *E. coli* may be cloned in *E. coli* K–12 under BL3 + EK1 containment conditions.
>
> *E. coli* host-vector systems expressing the Shiga-like toxin gene may be moved from BL3 to BL2 containment conditions provided that: (1) The amount of toxin produced by the modified host-vector systems is no greater than that produced by the positive control strain 933 *E. coli* 0157H7, grown and measured under optimal conditions; and (2) the cloning vehicle is to be an EK1 vector preferably belonging to the class of poorly mobilizable plasmids such as pBR322, pBR328, and pBR325.
>
> Nontoxinogenic fragments of the Shiga-like toxin structural gene(s) may be moved from BL3 + EK1 to BL2 + EK1 containment conditions or such nontoxic fragments may be directly cloned in *E. coli* K–12 under BL2 + EK1 conditions provided that the *E. coli* host-vector systems containing the fragments do not contain overlapping fragments which together would encompass the Shiga-like toxin structural gene(s).

Dr. O'Brien is now requesting permission to clone SLT structural genes, defined either by nucleotide sequence homology with SLT gene probes from *E. coli* or by antigenic cross-reactivity of their gene products with purified *E. coli* SLT, from bacterial species classified in the families *Enterobacteriaceae* or *Vibrionaceae* into *E. coli* K–12 under conditions similar to those specified in the previous permission. Dr. O'Brien wishes to broaden the scope of study of SLT toxin for the following reasons: (1) To investigate the potential role of SLT in the pathogenicity of a variety of bacteria; (2) to compare the SLT genes from various pathogenic bacteria; and (3) to facilitate the development of an experimental *Vibrio cholerae* vaccine strain deleted for SLT genes.

Dr. O'Brien has supplied information supporting this request to ORDA.

II. Request To Clone a Hybrid Toxin Gene in E. Coli K–12

Dr. John Murphy of the University Hospital of Boston University Medical Center requests permission to construct a hybrid molecule in which the gene coding for interleukin-2 (IL2) is joined to a segment of the gene encoding diphtheria toxin. The diphtheria toxin segment encodes the A subunit and portions of the B subunit. The hybrid gene would be cloned in *E. coli* K–12 host-vector systems.

III. Proposed Amendments of Appendix C–III of the NIH Guidelines

BioTechnica International, Cambridge, Massachusetts, proposes the following amendments to Appendix C–III of the NIH Guidelines. The first paragraph of Appendix C–III would be amended to read as follows:

Experiments which use *Saccharomyces cerevisiae* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines.

This amendment would extend the current exemption to include strains other than "laboratory strains."

A new second paragraph would be added to Appendix C–III to read as follows:

Experiments which use *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines.

The company has provided information concerning this request to ORDA.

IV. Points To Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols

Proposed "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" were published for comment in the Federal Register of January 22, 1985 (50 FR 2940). Readers are referred to that announcement for detailed background information and the roster of the RAC Working Group on Human Gene Therapy. Fifteen letters were received during the comment period. Based on those comments, the RAC Working Group on Human Gene Therapy met on April 1, 1985, and prepared a revised proposed Points to Consider. The revised proposed Points to Consider and letters of comment were reviewed by the full RAC at its meeting on May 3, 1985. After extensive discussion, the RAC voted to incorporate changes suggested at the meeting, to accept the revised Points to Consider as a working document, and to republish the document for further comment. An additional section, entitled "Applicability," has been added to emphasize that the Points to Consider apply only to institutions receiving support for recombinant DNA research from the NIH.

The following Points to Consider document incorporating the recommended changes is now published for public comment. Comments received will be circulated to all RAC and working group members. The Points to Consider document and the public comments received will be considered at the next RAC meeting on September 23, 1985.

NATIONAL INSTITUTES OF HEALTH POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF HUMAN SOMATIC-CELL GENE THERAPY PROTOCOLS

Working Group on Human Gene Therapy NIH Recombinant DNA Advisory Committee

Outline

Applicability
Introduction
I. Description of Proposal
  A. Objectives and Rationale of the Proposed Research
  B. Research Design, Anticipated Risks and Benefits
  1. Structure and Characteristics of the Biological System
  2. Preclinical Studies, Including Risk-Assessment Studies
  3. Clinical Procedures, Including Patient Monitoring
  4. Public-Health Considerations
  5. Qualifications of Investigators, Adequacy of Laboratory and Clinical Facilities
  C. Selection of Patients
  D. Informed Consent
  E. Privacy and Confidentiality
II. Special Issues
  A. Provision of Accurate Information to the Public
  B. Timely Communication of Research Methods and Results to Investigators and Clinicians
III. Requested Documentation
  A. Original Protocol
  B. IRB and IBC Minutes and Recommendations
  C. One-Page Abstract of Gene Therapy Protocol
  D. One-Page Description of Proposed Experiment in Non-Technical Language
  E. *Curricula vitae* for Professional Personnel
  F. Indication of Other Federal Agencies To Which the Protocol Is Being Submitted
  G. Other Pertinent Material
IV. Reporting Requirements

Applicability

These "Points to Consider" apply only to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from the National Institutes of Health (NIH). This includes research performed by NIH directly.

Introduction

(1) Experiments in which recombinant DNA [1] is introduced into cells of a human subject with the intent of stably modifying the subject's genome are covered by Section III–A–4 of the NIH Guidelines for Research Involving Recombinant DNA Molecules (49 FR 46266). Section III–A–4 requires such experiments to be reviewed by the NIH Recombinant DNA Advisory Committee (RAC) and approved by the NIH. RAC consideration of each proposal will be on a case-by-caes basis and will follow publication of a precis of the proposal in the Federal Register, an opportunity for public comment, and a review of the proposal by the working group of the RAC. RAC recommendations on each proposal will be forwarded to the NIH Director for a decision which will then be published in the Federal Register. In accordance with Section IV–C–1–b of the NIH Guidelines, the NIH Director may approve proposals only if he finds that they present "no significant risk to health or the environment."

(2) In general, it is expected that somatic-cell gene therapy protocols will not present a risk to the environment as the recombinant DNA is expected to be confined to the human subject. Nevertheless, Section I–B–4–b of the "Points to Consider" document asks the researchers to address specifically this point.

(3) This document is intended to provide guidance in preparing proposals for NIH consideration under Section III–A–4 of the NIH Guidelines for Research Involving Recombinant DNA Molecules. Not every point mentioned in the "Points to Consider" document will necessarily require attention in every proposal. It is expected that the document will be considered for revision at least annually as experience in evaluating proposal accumulates and as new scientific developments occur.

(4) A proposal will be considered by the RAC only after the protocol has been approved by the local Institutional Biosafety Committee (IBC) and by the local Institutional Review Board (IRB) in accordance with Department of Health and Human Services (DHHS) Regulations for the Protection of Human Subjects (45 CFR Part 46). If a proposal involves children, special attention should paid to subpart D of these DHHS regulations. The IRB and IBC may, at their discretion, condition their approval on further specific deliberation by the RAC and its working group. Consideration of gene therapy proposals by the RAC may proceed simultaneously with review by any other involved federal agencies [2] provided that the RAC

[1] Section III–A–4 applies to both recombinant DNA and DNA derived from recombinant DNA.

[2] The Food and Drug Administration (FDA) has jurisdiction over drug products intended for use in

Continued

is notified of the simultaneous review. Meetings of the committee will be open to the public except where trade secrets or proprietary information will be disclosed. The committee would prefer that the first proposal submitted for RAC review contain no proprietary information or trade secrets, enabling all aspects of the review to be open to the public. The public review of these protocols will serve to inform the public not only on the technical aspects of the proposals but also on the meaning and significance of the research.

(5) The clinical application of recombinant DNA techniques to human gene therapy raises two general kinds of questions: (1) The questions usually discussed by IRBs in their review of *any* proposed research involving human subjects; and (2) broader social issues. The first type of question is addressed principally in Part I of this document. Several of the broader social issues surrounding human gene therapy are discussed later in this Introduction and in Part II below.

(6) Following the Introduction, this document is divided into four parts. Part I deals with the short-term risks and benefits of the proposed research to the patient [3] to other people, as well as with issues of fairness in the selection of patients, informed consent, and privacy and confidentiality. In Part II, investigators are requested to address special issues pertaining to the free flow of information about clinical trials of gene therapy. These issues lie outside the usual purview of IRBs and reflect general public concerns about biomedical research. Part III summarizes other requested documentation that will assist the RAC and its working group in their review of gene therapy proposals. Part IV specifies reporting requirements.

(7) A distinction should be drawn between making genetic changes in somatic cells and in germ line cells. The purpose of somatic cell gene therapy is to treat an individual patient, e.g., by inserting a properly functioning gene into a patient's bone marrow cells *in vitro* and then reintroducing the cells into the patient's body. In germ line alterations, a specific attempt is made to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring. The RAC and its working group will not at present entertain proposals for germ line alterations but will consider for approval protocols involving somatic-cell gene therapy.

(8) The acceptability of human somatic-cell gene therapy has been addressed in several recent documents as well as in numerous academic studies. The November 1982 report of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, *Splicing Life*, resulted from a two-year process of public deliberations and hearings; upon release of that report, a House subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, *Human Gene Therapy*, which brought these earlier documents up-to-date. As the latter report concluded:

> Civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies.

(9) Concurring with this judgment, the RAC and its working group are prepared to consider for approval somatic-cell therapy protocols, provided that the design of such experiments offers adequate assurance that their *consequences* will not go beyond their *purpose*, which is the same as the traditional purpose of all clinical investigations, namely, to benefit the health and well-being of the individual being treated while at the same time gathering generalizable knowledge.

(10) The two possible undesirable consequences of somatic-cell therapy would be unintentional (1) vertical transmission of genetic changes from an individual to his or her offspring or (2) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, this document requests information that will enable the RAC and its working group to assess the likelihood that the proposed somatic-cell gene therapy will inadvertently affect reproductive cells or lead to infection of other people (e.g., treatment personnel or relatives).

(11) In recognition of the social concern that surrounds the general discussion of human gene therapy, the working group will continue to consider the possible long-range effects of applying knowledge gained from these and related experiments. While research in molecular biology could lead to the development of techniques for germ line intervention or for the use of genetic means to enhance human capabilities rather than to correct defects in patients, the working group does not believe that these effects will follow immediately or inevitably from experiments with somatic-cell gene therapy. The working group will cooperate with other groups in assessing the possible long-term consequences of somatic-cell gene therapy and related laboratory and animal experiments in order to define appropriate human applications of this emerging technology.

(12) Responses to the questions raised in these "Points to Consider" should be provided in the form of either written answers or references to specific sections of the protocol or its appendices.

I. Description of Proposal

A. Objectives and Rationale of the Proposed Research

State concisely the overall objectives and rationale of the proposed study. Please provide information on the following specific points:

1. Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?

2. Describe the natural history and range of expression of the disease selected for treatment. In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene therapy?

3. Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

4. What alternative therapies exist? In what groups of patients are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene therapy?

B. Research Design, Anticipated Risks and Benefits

1. Structure and Characteristics of the Biological System

Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

a. What is the structure of the cloned DNA that will be used?

(1) Describe the gene (genomic or cDNA), the bacterial plasmid or phage

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clinical trials of human somatic-cell gene therapy. For general information on FDA's policies and regulatory requirements, please see the Federal Register, Volume 49, pages 50878–80, 1984.

[3] The term "patient" and its variants are used in the text as a shorthand designation for "patient-subject."

vector, and the delivery vector (if any). Provide complete sequence analysis or a detailed restriction map of the total construct.

(2) What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)?

(3) Describe the steps used to derive the DNA construct.

b. What is the structure of the material that will be administered to the patient?

(1) Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells.

(a) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)? What tests have been used and what is the sensitivity of the tests?

(b) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (nucleic acids, proteins, etc.) or contaminating viruses or other organisms in the cells or serum?

(2) Describe any other material to be used in preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

2. Preclinical Studies, Including Risk-Assessment Studies

Describe the experimental basis (derived from tests in cultured cells and laboratory animals) for claims about the efficacy and safety of the proposed system for gene delivery.

a. *Laboratory studies of the delivery system.* (1) What cells are the intended recipients of gene therapy? If recipient cells are to be treated *in vitro* and returned to the patient, how will the cells be characterized before and after treatment? What is the theoretical and practical basis for assuming that only the treated cells will act as recipients?

(2) Is the delivery system efficient? What percentage of the target cells contain the inserted DNA?

(3) How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

(4) How many copies are inserted per cell? How stable is the inserted DNA both in terms of its continued presence and its structural stability?

b. *Laboratory studies of gene expression.* Is the inserted gene expressed? To what extent is expression only from the desired gene (and not from the surrounding DNA)? In what percentage of cells does expression occur? Is the product biologically active? What percentage of normal activity results from the inserted gene? Is the gene expressed in cells other than the target cells? If so, to what extent?

c. *Laboratory studies pertaining to the safety of the delivery/expression system.* (1) If a retroviral system is used:

(a) What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

(b) How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

(c) What laboratory evidence is available concerning potential harmful effects of the treatment, e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses? What steps have been taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

(d) Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ line cells? What is the sensitivity of the analyses?

(e) Has a protocol similar to the one proposed for a clinical trial been carried out in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

(2) If a non-retroviral delivery system is used: What animal studies have been done to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ line cells)? How long have the animals been studied after treatment? What tests have been used and what is their sensitivity?

3. *Clinical Procedures, Including Patient Monitoring*

Describe the treatment that will be administered to patients and the diagnostic methods that will be used to monitor the success or failure of the treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study.

a. Will cells (e.g., bone marrow cells) be removed from patients and treated *in vitro* in preparation for gene therapy? If so, what kinds of cells will be removed from the patients, how many, how often, and at what intervals?

b. Will patients be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy) prior to gene therapy?

c. What treated cells (or vector/DNA combination) will be given to patients in the attempt to administer gene therapy? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple treatments? If so, over what period of time?

d. What are the clinical endpoints of the study? How will patients be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be done? How long will patient follow-up continue?

e. What are the major potential beneficial and adverse effects of treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of potential adverse effects on patients with the probability and magnitude of deleterious consequences from the disease if gene therapy is not performed.

f. If a treated patient dies, what special studies will be performed as part of the autopsy?

4. Public-Health considerations

Describe any potential benefits and hazards of the proposed therapy to persons other than the patients being treated. Specifically:

a. On what basis are potential public health benefits or hazards postulated?

b. Is there a significant likelihood that the inserted DNA will spread from the patient to other persons or to the environment?

c. Will any precautions be taken against such spread (e.g., to patients

sharing a room, health-care workers, or family members)?

d. What measures will be undertaken to mitigate the risks, if any, to public health?

5. Qualifications of Investigators, Adequacy of Laboratory and Clinical Facilities

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of gene therapy. In addition, please describe the laboratory and clinical facilities where the proposed study will be performed.

a. What professional personnel (medical and nonmedical) will be involved in the proposed study? What are their specific qualifications and experience with respect to the disease to be treated and with respect to the techniques employed in molecular biology? Please provide *curricula vitae* (see Section III–D).

b. At what hospital or clinic will the treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will patients occupy regular hospital beds or clinical research center beds? Where will patients reside during the follow-up period?

C. Selection of Patients

Estimate the number of patients to be involved in the proposed study of gene therapy. Describe recruitment procedures and patient eligibility requirements, paying particular attention to whether these procedures and requirements are fair and equitable.

1. How many patients do you plan to involve in the proposed study?

2. How many eligible patients do you anticipate being able to identify each year?

3. What recruitment procedures do you plan to use?

4. What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

5. How will patients be selected if it is not possible to include all who desire to participate?

D. Informed Consent

Indicate how patients will be informed about the proposed study and how their consent will be solicited. The consent procedure should adhere to the requirements of DHHS regulations for the protection of human subjects (45 CFR Part 46). If the study involves pediatric or mentally handicapped patients, describe procedures for seeking the permission of parents or guardians and, where applicable, the assent of each patient. Areas of special concern highlighted below include potential adverse effects, financial costs, privacy, and long-term follow-up.

1. How will the major points covered in Sections I–A through I–C of this document be disclosed to potential participants in this study and/or parents or guardians in language that is understandable to them?

2. How will the innovative character and the theoretically-possible adverse effects of gene therapy be discussed with patients and/or parents or guardians? How will the potential adverse effects be compared with the consequences of the disease? What will be said to convey that some of these adverse effects, if they occur, could be irreversible?

3. What explanation of the financial costs of gene therapy and any available alternative therapies will be provided to patients and/or parents or guardians?

4. Will patients and/or their parents or guardians be informed that the innovative character of gene therapy may lead to great interest by the media in the research and in treated patients? What special procedures, if any, will be followed to protect the privacy of patients and their families?

5. Will patients and/or their parents or guardians be informed:

a. That some of the procedures performed in the study will be irreversible.

b. That following the performance of such procedures it would not be medically advisable for patients to withdraw from the study?

c. That a willingness to cooperate in long-term follow-up (for at least three to five years) will be a precondition for participation in the study?

d. That a willingness to permit an autopsy to be performed in the event of a patient's death following treatment is also a precondition for a patient's participation in the study? (This stipulation is included because an accurate determination of the precise cause of a patient's death would be of vital importance to all future gene therapy patients.)

E. Privacy and Confidentiality.

Indicate what measures will be taken to protect the privacy of gene therapy patients and their families as well as to maintain the confidentiality of research data.

1. What provisions will be made to honor the wishes of individual patients (and the parents or guardians of pediatric or mentally handicapped patients) as to whether, when, or how the identity of patients is publicly disclosed?

2. What provision will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual patients?

II. Special Issues

Although the following issues are beyond the normal purview of local IRBs, the RAC and its working group request that investigators respond to questions A and B below.

A. What steps will be taken to ensure that accurate information is made available to the public with respect to such public concerns as may arise from the proposed study?

B. Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among investigators and clinicians concerning research methods and results?

III. Requested Documentation

In addition to responses to the questions raised in these "Points to Consider," please submit the following materials:

A. Your protocol as approved by your local IRB and IBC. The consent form, which must have IRB approval, should be submitted to the NIH only on request.

B. Local IRB and IBC minutes and recommendations that pertain to your protocol.

C. A one-page scientific abstract of the gene therapy protocol.

D. A one-page description of the proposed experiment in nontechnical language.

E. *Curricula vitae* for professional personnel.

F. An indication of other federal agencies to which the protocol is being submitted for review.

G. Any other material which you believe will aid in the review.

IV. Reporting Requirements

A. Serious adverse effects of treatment should be reported immediately to both your local IRB and the NIH Office for Protection from Research Risks, and a written report should be filed with both groups. A copy of the report should also be forwarded to the NIH Office of Recombinant DNA Activities (ORDA).

B. Reports regarding the general progress of patients should be filed at six-month intervals with both your local IRB and ORDA. These twice-yearly reports should continue for a sufficient period of time to allow observation of major effects (at least three to five

years). In the event of a patient's death, the autopsy report should be submitted to the IRB and ORDA.

Dated: August 9, 1985

James B. Wyngaarden,

Director, National Institutes of Health

[OMB's "Mandatory Information requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.]

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE
SUBWORKING GROUP ON HUMAN GENE THERAPY

MINUTES OF MEETING[1]

September 20, 1985

The Subworking Group on Human Gene Therapy of the Recombinant DNA Advisory Committee was convened at 9:00 a.m. on September 20, 1985, at the National Institutes of Health, Building 31C, Conference Room 9, 9000 Rockville Pike, Bethesda, Maryland 20892. The meeting was open to the public. Dr. LeRoy Walters was the Chair. The following people were present for all or part of the meeting:

Subworking Group Members:

W. French Anderson	Robert Murray
Judith Areen	LeRoy Walters
James Childress	Anne Witherby
Susan Gottesman	William Gartland, Jr.
Robert Mitchell	(Executive Secretary)

A working group roster is attached (Attachment I).

Other National Institutes of Health Staff:

Elizabeth Milewski, NIAID

[1]The subworking group is advisory to the RAC, and its recommendations should not be considered as final or accepted.

Dr. Walters called the meeting of a Subworking Group on Human Gene Therapy to order at 9:10 a.m. on September 20, 1985. Dr. Walters said the subworking group would be dealing with three agenda items: (1) a review of the comments received on the August 19, 1985, Federal Register version of the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy (Attachment II);" (2) a discussion of the optimal presentation of the "Points to Consider" to the Recombinant DNA Advisory Committee (RAC) at the September 23, 1985, meeting; and (3) a discussion of date(s) and agenda(s) for future meetings of the Working Group on Human Gene Therapy.

Dr. Walters called the attention of the subworking group to a letter (Attachment III) from Senator Albert Gore to the Secretary of Health and Human Services (HHS) Margaret Heckler. Senator Gore said he was "writing to express his concern over the current effort of the Food and Drug Administration and the proposed Biotechnology Science Board to usurp the role of the National Institutes of Health Recombinant DNA Advisory Committee in overseeing human gene therapy experiments." He wrote he was opposed to this effort and called upon Secretary Heckler to end it immediately. Senator Gore wrote that the Recombinant DNA Advisory Committee (RAC) has adequately addressed the scientific issues to date, and it appears capable of continuing to do so for the immediate future.

Review of Comments on August 1985 Version of Points to Consider

Dr. Walters pointed out to the subworking group the differences between the August 19, 1985, version of the points to consider document (Attachment II) and the May 3, 1985, version: (1) Language indicating the applicability of the points to consider has been added to the document. (2) The first footnote has been amended to indicate the document refers to recombinant DNA and DNA derived from recombinant DNA. (3) A paragraph was deleted from the Introduction; that paragraph indicated the document is designed to cover the initial human somatic cell gene therapy protocols and that initial protocols are expected to involve only one or a few patients at a time. (4) A footnote describing the role of the Food and Drug Administration (FDA) was added to the document. (5) In the tenth paragraph of the Introduction the word "possible" had been substituted for the word "primary" in referring to consequences of somatic cell therapy. (6) A sentence indicating it is likely that possible undesirable side effects can be prevented was deleted from the document. (7) A sentence requesting that the consent form routinely be included as part of the submission has been deleted from this version of the points to consider document at the request of the National Institutes of Health (NIH) legal counsel since NIH does not routinely request consent forms. A sentence indicating the working group may request submission of the consent form in certain cases has been added to Section III-A.

Mr. Mitchell asked how the language describing FDA's role had been developed. Dr. Gartland replied that FDA legal counsel had developed that language.

Mr. Mitchell said questions had been raised at the May 3, 1985, RAC meeting concerning adequately distinguishing between the consent form and the consent

process. He said the subworking group might consider whether the points to consider document adequately makes this distinction.

Dr. Murray said currently investigators must describe to the Institutional Review Board (IRB) the method of selecting the subject(s). Investigators must also provide information on how the patient will be informed of the procedure. The IRB submits assurances to DHHS on this process. The IRB determines whether the information and process are appropriate but does not have a mechanism to monitor the consent process.

Mr. Mitchell said a whole body of law is developing for situations in which an individual surrenders fundamental rights. This body of law attempts to ensure that the individual fully understands and can make a knowledgeable decision concerning these rights. He offered as examples individuals entering a guilty plea or placing a child in adoption procedures. A similar body of law may be developing for informed consent in clinical trials.

Dr. Walters noted that the language added to Section III-A permits the working group to request the consent form in some circumstances. He said he would have preferred the points to consider indicate the working group would review consent form(s) as part of the review process. Dr. Anderson agreed it is illogical for the points to consider to contain a whole section on informed consent but not to request the form.

Dr. Walters asked whether NIH study sections routinely receive consent forms with grant applications.

Dr. Anderson said study sections receive consent forms, but they are concerned primarily with scientific and technical issues and not with the informed consent process. Dr. Murray said submission of the consent form with the grant application ensures the grant proposal was reviewed by the IRB before submission for NIH review.

Dr. Gottesman suggested the NIH legal advisor did not wish an NIH advisory group to be responsible for reviewing consent forms since such review might involve the NIH in liability issues. Dr. Walters suggested some risk liability might, nonetheless, exist for NIH in performing the scientific risk/benefit analysis.

Mr. Mitchell said he could understand the legal advisor's concern since a body of law regarding informed consent has not yet been developed. The subworking group agreed the points to consider should not at this time contain language requiring submission of the consent form.

Dr. Gartland suggested the working group should discuss informed consent issues with individuals such as Dr. Charles McCarthy of the NIH Office for Protection from Research Risks (OPRR) and Mr. Robert Lanman, the NIH Legal Counsel. Dr. Gottesman suggested a meeting of the working group with these individuals be arranged.

Dr. Walters then called the attention of the subworking group to comments on the August 1985 version of the points to consider document offered by Dr. Howard Temin, a consultant to the Working Group on Human Gene Therapy.

Dr. Walters said Dr. Temin had once again pointed out that the vectors most likely to be used in human gene therapy protocols are retroviruses. The genetic material of these viruses is RNA. Dr. Temin suggested some mention of recombinant RNA should be made in the first footnote of the points to consider document.

Mr. Mitchell said RAC at the May 3, 1985, meeting had discussed the possibility of modifying the NIH Guidelines to explicitly cover recombinant RNA. The subworking group agreed that until such action is taken the first footnote should refer only to recombinant DNA.

Dr. Walters said Dr. Temin had also suggested Section I-B-1-a-(1) should be modified to read as follows:

> "Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme cleavage site map of the total construct."

Dr. Gottesman said the section as written contains scientific jargon; she thought Dr. Temin's suggested language was more specific. Dr. Walters agreed Dr. Temin's suggested language was more specific but thought it used many nouns as adjectives.

Dr. Anderson suggested the subworking group accept most of Dr. Temin's suggested language but not the words "cleavage site." The language would then be more precise but not use so many nouns as adjectives. The subworking group agreed to this modification.

Dr. Walters said Dr. Temin had suggested the last sentence of Section I-B-1-b-(2) should read as follows:

> "What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (nucleic acids, proteins, etc.) or contaminating viruses or other organisms in the cells or serum used for preparation of the virus stock?"

The subworking group agreed to this suggested clarification.

Dr. Walters said Dr. Temin had suggested the word "added" be substituted for the word "inserted" in Section I-B-2-a-(2), Section I-B-2-a-(4), Section I-B-2-b, and Section I-B-4-b.

Dr. Anderson admitted the word "inserted" is ambiguous, but felt the word "added" is also ambiguous. He and Dr. Gottesman noted that "added" could also refer to DNA which is simply attached to the cells' outer membrane and not introduced into the cytoplasm or the nucleus.

Dr. Gottesman said the word "added" promises less than the word "inserted;" for this reason the word "added" might be preferable in certain sections of the document since it would apply even if the DNA had not inserted in the chromosome.

The subworking group agreed to substitute the word "added" in Section I-B-1-a-(2), in the second sentence of Section I-B-1-a-(4), in the first sentence of Section I-B-1-b, and in Section I-B-4-b. They suggested the word "present" be substituted for the word "added" in the first sentence of Section I-B-1-a-(4).

Dr. Walters said Dr. Temin had also suggested the second sentence of Section I-B-2-b should read as follows:

"In what percentage of cells does expression occur only from the added DNA?"

Dr. Gottesman thought this concept was included in the second sentence of Section I-B-2-b. She suggested this sentence could be modified to more clearly state this idea and would read as follows:

"In what percentage of cells does expression from added DNA occur?"

The subworking group agreed to Dr. Gottesman's suggested modification.

Dr. Walters said Dr. Temin had also suggested a new sentence be added to Section I-B-2-b as follows:

"In what percentage of cells does expression occur from other DNA sequences as a result of the added DNA?"

Dr. Anderson questioned how one would test whether expression from other genes occurs as a result of inserting the vector in the chromosome. He did not think it currently feasible to test for such expression.

The subworking group agreed the points to consider should not request information on whether other genes are expressed if a vector inserts in the genetic material.

Dr. Walters then called the attention of the subworking group to the letter (Attachment IV) from Mr. Edward Lee Rogers on behalf of the Foundation on Economic Trends.

Mr. Mitchell said the first comment offered by Mr. Rogers is that frequent revisions of the points to consider may be needed as experience is gained in this field. Mr. Rogers did not believe the points to consider should assume how soon or how often revisions may be necessary.

The subworking group agreed with this comment. Dr. Walters said the points to consider should convey the idea that the working group will revise the document as needed; if revision is necessary more than once a year, the document will be revised more frequently.

Dr. Gottesman said the language of paragraph (3) of the Introduction to the points to consider document could better express this intent if modified as follows:

> "The document will be considered for revision as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out at least annually."

The subworking group agreed to this modification.

Dr. Walters said Mr. Rogers also suggested the working group begin immediately to develop the procedural structure for cooperative efforts in assessing possible long-term consequences of somatic-cell gene therapy. The subworking group said the Working Group on Human Gene Therapy is committed to cooperative efforts.

Dr. Walters said Mr. Rogers had pointed out a typographical error in Section I-B-5-a; the language referring to Section III-D should refer to Section III-E. The subworking group agreed this error should be corrected.

Dr. Walters said Mr. Rogers had also suggested the working group should review its own composition. Dr. Walters said the Working Group on Human Gene Therapy had already discussed the issue of the working group's composition and is committed to seeking the opinions of consultants who can provide the requisite scientific and social expertise.

Dr. Anderson said Mr. Rogers also suggested Section I-B-5 of the points to consider should list experts in disciplines such as bioethics as "nonmedical" personnel.

Mr. Mitchell said the working group should maintain the prerogative of determining on a case-by-case basis which expertise should be represented on the research team. The subworking group agreed and rejected this proposal.

Dr. Childress said Mr. Rogers also alludes to the issue of whether there are "right" answers to some of the points to consider. Dr. Childress asked whether the working group might at some point move towards determining what would constitute "right" answers.

Dr. Walters agreed some of the points are rhetorical. The working group in some points is simply asking "have you thought about it." At this time, the working group cannot indicate how it will respond to particular issues.

Dr. Anderson said human gene therapy is a novel treatment process; in any new endeavor it is difficult to define what will be required since it is difficult to determine what is important without experience. Dr. Walters suggested a consensus may develop spontaneously in the field as experience accumulates. Changes will be made in the document when experiences suggests modifications are appropriate.

Dr. Anderson said the thinking regarding the appropriate initial patient and the appropriate approach has recently been changing. Some clinicians now feel a subject who can be helped by other options should be chosen rather than a patient for whom gene therapy is the last hope. At this time, an infant who has two years or more to live and for whom a backup therapy exists is the preferred candidate, in his view. For the first subjects, gene therapy should be the most promising of the available therapies; this situation would show whether or not the patient has been helped by the therapy.

Dr. Anderson added that when his team actually came to the point of "walking through" a gene therapy protocol with primates, they encountered technical and logistical problems which were impossible to anticipate from experience with mice. To date, most researchers have performed gene transplants with mice; mice do not present the same types of logistical problems large animals present.

Dr. Anderson said although his group has a great deal of experience with bone marrow transplants in mice, everything that technically could go wrong went wrong when they performed the procedure on monkeys. The procedure did not work in the first four monkeys; as experience has accumulated, however, the probability of success has increased. Experience with a large animal such a dog, pig, or primate is very important.

Dr. Anderson said the "gene team" approach to gene therapy appears to be the optimal approach because of the logistical problems associated with the therapy; scientists who prepare retroviral vectors will work with experts who have experience in handling large amounts of bone marrow and clinicians experienced in bone marrow transplantation. His team at the NIH will perform gene therapy on four monkeys a month every month to maintain the necessary degree of familiarity with the procedure. Gene therapy teams need experience with large animals.

Ms. Witherby asked whether the working group document should mention this obvious fact. Dr. Anderson did not think the document needed to mention this fact.

Dr. Murray suggested the subworking group reiterate the statement that the document will be refined as experience is gained.

Dr. Gottesman said Mr. Roger's final comment is that the working group should develop criteria in Part II (Special Issues) rather than simply ask questions. The subworking group agreed it is preferable at this time to simply ask investigators to think about these issues.

Dr. Childress said a tension exists in the document between the patient's right to privacy and the public's right to know. He said the working group would give primacy to the privacy of the patient and the family; the working group does not care if the public is interested in the identity of the child.

Dr. Areen suggested the portion of the points to consider referring to the right of the public to know should be cross referenced to the section dealing with privacy and confidentiality issues.

The subworking group agreed Section II-A should read as follows:

> "What steps will be taken, consistent with point I-E above, to ensure that accurate information is made available to the public with respect to such public concerns as may arise from the proposed study?"

Dr. Walters asked whether it would be appropriate for the subworking group to respond to Mr. Rogers' letter. The subworking group agreed a letter to Mr. Rogers would be appropriate.

Mr. Mitchell and Dr. Childress suggested a letter of commendation and thanks might be sent to Dr. Temin for his invaluable assistance as a consultant.

Dr. Walters reported that Dr. Gartland had solicited the opinion (Attachment V) of Dr. Myron Max Levine of the University of Maryland, a former RAC member and a specialist in vaccine development. Dr. Levine had written that the points to consider are excellent, balanced, conservative, and sensitive to social issues. Dr. Levine felt a willingness to cooperate in long-term follow-up and to permit an autopsy should be prerequisites for participation in the earliest clinical trials. The subworking group agreed these procedures should be required of the initial patients.

Dr. Gartland said the NIH legal counsel would prefer Section I-D-5 not be included as part of Section I-D since Section I-D-1 through Section I-D-4 are questions while Section I-D-5 contains a statement.

Dr. Childress said Section I-D inquires how the privacy of the patient will be protected; Section I-D-5 which deals with autopsy. Follow-up could be placed in other sections of the document as well as in Section I-D.

Dr. Walters thought Section I-D-5 dealt with points which the working group considers to be particularly important in human gene therapy and which should be emphasized in informed consent.

Dr. Gottesman suggested Sections I-D-4 and I-D-5 should be modified to ask "how" patients will be informed. A question mark will be added to Section I-D-5-a. The subworking group agreed.

Dr. Childress suggested the second sentence of Section I-D-3 should be deleted since it is not really a part of informed consent. That sentence reads as follows:

"What special procedures, if any, will be followed to protect the privacy of patients and their families?"

The subworking group agreed to delete this sentence.

Dr. Walters then called the attention of the subworking group to the September 13, 1985, comments (Attachment VI) of Dr. Henry Miller of the FDA. In his first comment, Dr. Miller suggested the points to consider should state that closed sessions will be available for review of human gene therapy protocols. Dr. Gottesman said this comment had been considered by the Working Group on Human Gene Therapy at the April 1985 meeting; the points to consider document had been modified by the working group in response to this comment at that time. While the working group will not refuse to hold closed sessions, it did not wish to encourage closed session review; and the points to consider reflect this position.

Dr. Walters said Dr. Millers's second comment was that the points to consider ignore that the appropriateness of medical therapies is the result of complex risk/benefit judgments; Dr. Miller thought the points to consider imply that any risk of teratogenesis is unacceptable.

Dr. Gottesman said a complex risk/benefit judgment determines the appropriateness of any therapy, and this is also true of human gene therapy. In human gene therapy two potential risks exist: (1) vertical transmission of genetic material; and (2) horizontal transmission of genetic material. Both of these potential risks must be considered; the examples offered by Dr. Miller do not consider horizontal transmission.

Dr. Murray said the word "teratogenesis" is not appropriately used by Dr. Miller; Dr. Miller intends to refer to transmission of genetic information. Dr. Murray said transmission of genetic information is not necessarily teratogenic.

Dr. Anderson said clinicians recognize that inadvertent transmission of genetic information might occur in gene therapy; however, transmission would not be the goal of the therapy.

Mr. Mitchell said the working group drafted the document to allay public fears about germ line intervention but is aware 100% assurance against inadvertent transmission cannot be given.

Dr. Childress asked whether the document should state explicitly that some risk of inadvertent transmission to germ line cells would be acceptable.

Dr. Gottesman thought mention of inadvertent transmission in this context would raise a red flag. She thought clinicians and scientists implicitly recognize inadvertent transmission might occur.

Dr. Murray agreed; he said the working group document does not take a position on whether "inadvertent changes" in the patient's germ line are permissible. Dr. Murray said the treatment of children with leukemia offers a precedent. The effect of chemotherapy on these patients' offspring will not be known until these patients have children. Most clinicians conclude, however, a fair possibility exists that the germ line cells of these patients will be affected. Nonetheless, in childhood leukemia therapy, the risk of damaged offspring is balanced against the benefit of prolonging the life of the patient. The Working Group on Human Gene Therapy is asking for the data to make the same type of risk/benefit analysis. He felt the working group was on firm ground as long as it evaluated potential inadvertent effects and not intended changes in the germ line. Dr. Walters agreed.

Dr. Anderson said the initial protocols will involve use of modified bone marrow cells. Added DNA does not appear to be transferred from these cells to germ line cells, and effects on germ line cells are not likely.

Ms. Areen suggested the working group might communicate to Dr. Miller that it has taken no position on whether inadvertent modification of the germ line is good or bad.

Dr. Anderson suggested the word "the" should be deleted from the tenth paragraph of the Introduction of the points to consider. The subworking group agreed.

Dr. Walters said Dr. Miller's third comment suggested the first sentence in Section I-B-1-b would be more clear if it referred to the "composition" of the material.

Dr. Gottesman said the working group had considered this comment at an earlier meeting. The term "composition" is inappropriate in the context of human gene therapy protocols because composition refers to the chemical composition of a substance. The subworking group agreed.

Dr. Walters then said Dr. Miller's fourth comment suggested two new subsections be added. One of the new subsections would ask the investigator to "describe in detail the methods for harvesting, extraction, and purification and for the removal of any toxic chemicals introduced by these procedures." The second new subsection would contain a warning that penicillin and other beta-lactam antibiotics should not be used in the production of materials administered to patients.

Dr. Gottesman said the working group had considered these two proposed subsections at the April 1985 meeting. The terms "harvesting, extraction, and purification" do not apply to the initial human gene therapy procedures; the points to consider document contains the appropriate analogous language.

Dr. Anderson said the proposed subsection on beta-lactam antibodies is not relevant to human gene therapy procedures since the first clinical protocols will probably be based on procedures using modified bone marrow cells. These cells will be removed from the patient, modified, washed, and returned to the patient. Antibiotics which could cause an allergic reaction will not be present in the materials administered to the patient.

Dr. Gottesman said Dr. Miller's absolute statement prohibiting the use of beta-lactam antibiotics does not make sense since it does not specify when these antibiotics may not be used. This prohibition could be interpreted as applying to steps in the preparation of the retroviral vector in which an antibiotic might be necessary.

Dr. Walters said Dr. Miller's fifth suggestion was to add to Section I-B-1-b-(1) questions on the methods for assaying the potency of the product, the consistency of the product lot-by-lot, and the stability of the product under conditions of storage.

Dr. Murray said this proposed language had also been considered at the April 1985 meeting; at this time, it makes no sense to assume the initial clinical trials will involve commercial production. The initial gene therapy protocols will be administered on a patient-by-patient basis.

Dr. Anderson said Dr. Miller's proposed language applies in FDA reviews of drugs and biologics; however, the Working Group on Human Gene Therapy is not attempting to perform an FDA review.

Dr. Gartland asked whether the working group would review a proposal differently if FDA does not review the proposal. FDA statutes do not apply in all cases; for example FDA statutes do not apply to protocols which do not involve interstate commerce.

Dr. Gottesman said she viewed Dr. Miller's intimation that FDA might not review protocols as a threat the working group might be required to perform an FDA review. Dr. Gottesman said an FDA style review is not the working group's mandate or interest.

Drs. Anderson and Gottesman then explained that the words "potency," "consistency," and "stability" are appropriate language for drugs and biologicals but are not the correct terms to apply to human gene therapy. Genes do not have "potency," Rather the analogous term for a gene is "level of expression." The analogous information request for "stability" in gene therapy protocols is a request for information on the cell line which will be producing the retroviral vector. Cell lines producing retroviral vectors are stored in liquid nitrogen and are stable indefinitely under these conditions. "Expression in time" is the appropriate analogous term for "stability" of a gene. The language pertinent to genes is found in detail in the working group document.

Dr. Anderson said patients' bone marrow cells, which are critical to the procedure, will not be stored. He compared bone marrow transplants in human gene therapy to other organ transplantation. He said it makes no more sense to

inquire about "stability" in human gene therapy than it would to ask about the lot stability of a kidney in a transplant operation.

Dr. Walters asked if a patient might receive a second course of gene therapy.

Dr. Anderson said it is conceivable a patient might require a second course of therapy. However, each course will be a de novo procedure, and lot consistency will not be relevant.

Dr. Walters said Dr. Miller's sixth comment was that the working group document should request a description of previously-reported similar human studies (including foreign studies) and their results.

Dr. Walters said Dr. Miller had previously made this comment to the Working Group on Human Gene Therapy. Dr. Milewski pointed out that the working group at the April 1985 meeting had added language to Section I-B-3 in response to this comment.

Dr. Walters said Dr. Miller's seventh suggestion is that Section I-B-2-c-(1)-(d) should be relocated to a position before Section I-B-2-c-(1) since Section I-B-2-c-(1)-(d) is relevant to gene therapy whether or not a retroviral system is used.

Dr. Anderson said Section I-B-2-c-(1)-(c) is appropriately placed in the document since Section I-B-2-c-(1)-(c) specifically refers to the inherent properties of retroviral vectors. He said the key question is whether the vector has sequences homologous with human sequences. Drs. Varmus and Temin specifically placed Section I-B-2-c-(1)-(d) in its current position in the document.

The subworking group agreed the language was appropriately placed in Section I-B-2-c-(1)-(d).

Dr. Anderson said the working group could consider adding parallel language to Section I-B-2-c-(3); this section deals with other host-vector systems. He could not envisage, however, that this addition would be useful since it would address pathogenicity. He did not think plasmid vectors would possess pathogenic qualities.

Dr. Gottesman said while retroviruses have a mechanism to express pathogenicity, there is no evidence to suggest plasmid vectors might possess pathogenicity mechanisms. It is, thus, not logical to pose a question in Section I-B-2-c-(3) referring to plasmid pathogenicity. If a potential pathogenicity mechanism cannot be envisaged for plasmid vectors, how could the vector be designed to avoid the consequences of pathogenicity? She said the current points to consider document is inclusive and adequately addresses the pertinent issues. The subworking group agreed.

Dr. Walters said Mr. Miller's eighth suggestion is that the phrase "specifically germ line cells" should be deleted from Section I-B-2-c-(2) because apprehension

about effects on gametes should be not greater than apprehension for effects on other critical tissues.

Dr. Walters said to ignore this issue is to ignore a source of public apprehension. Ms. Areen said Dr. Miller had offered this comment to the working group in April 1985; the working group had substituted the word "particularly" for the word "specifically" in Section I-B-2-c-(2).

Dr. Anderson said Dr. Miller was expressing a private opinion in this comment. Dr. Anderson thought germ line effects are legitimate public concerns. The subworking group agreed.

Dr. Walters then called the attention of the subworking group to Dr. Miller's ninth comment. Dr. Miller had suggested the first sentence in Section I-B-2-c-(1)-(e) would be more clear if revised to read: "Describe animal experiments completed or in progress employing protocols similar to that proposed."

Dr. Anderson said the working group had also previously considered this comment and had elected not to modify the language of the points to consider document. The working group specifically wished to request data gathered from primate testing. Dr. Anderson said the experience of his research team emphasizes the importance of testing gene therapy protocols in large animals such as primates. Dr. Anderson asked whether Section I-B-2 should include a statement on testing in "a large animal."

Dr. Gottesman said a statement on animal testing is included in Section I-B-2. She suggested the word "laboratory" be deleted from this section since the phrase "laboratory animal" to most people suggests mice or rats. Deleting this word might meet part of Dr. Anderson's concern. The subworking group agreed with this proposal.

Dr. Gottesman noted that Dr. Miller had suggested the term "in progress" be added to the language of Section I-B-2-c-(1)-(e). She questioned whether Dr. Miller's proposed language would suggest review would be deferred to await the results of "in progress" experiments.

Ms. Areen suggested the language of the section was correct as currently written. The subworking group agreed the language as written was appropriate.

Dr. Walters said Dr. Miller's tenth comment was that "selection of subjects" should be included in Section I-B-3 rather than be a separate Section I-C.

Mr. Mitchell said the working group disagreed with Dr. Miller on the emphasis which should be given this topic.

Dr. Walters said the working group also wished to include ideas of fairness and equity and for this reason made "selection of subjects" a separate section.

Dr. Childress said the working group had rejected this comment at an earlier meeting, and the subworking group should reject it this time as well.

Dr. Walters then called the attention of the subworking group to Dr. Miller's eleventh comment. Dr. Miller had suggested the first sentence of Section I-B-3 is sufficient; the other sentences require extremely complex speculation of the investigator and should be deleted.

Mr. Mitchell said Section I-B-3 is not requesting speculation since in many cases the answer will be based on experience.

Dr. Murray said the second question in Section I-B-3 does not require speculation. In some cases, a great deal of clinical literature exists; this literature could suggest potential effects.

Dr. Anderson said a complex risk/benefit analysis is exactly what a clinician performs when treating patients. This is not speculation. The subworking group rejected Dr. Miller's eleventh comment.

Dr. Walters then called the attention of the subworking group to Dr. Miller's twelfth comment. Dr. Miller had stated that in Section I-D, the initial paragraph under informed consent would provide sufficient guidance if points 1 through 5 were deleted and the following language were added to the paragraph:

> "The consent form must adhere to the requirements of 45 CFR 46 and 21 CFR 50. Special attention must be paid to the applicability of the additional elements of informed consent listed in these regulations. (Include a copy of the patient consent form as part of the documentation requested in Part III, below.)"

The subworking group agreed the working group had discussed this suggestion at the April 1985 meeting and had determined it wished to highlight specific points. The subworking group agreed no change was indicated at this time.

Dr. Walters said Dr. Miller's thirteenth comment was that in Section I-E the initial paragraph under "Privacy and Confidentiality" would provide sufficient information without points 1 and 2; points 1 and 2 should be deleted.

Ms. Areen felt the working group document highlighted certain important issues. She felt this section of the document should not be altered.

Dr. Anderson agreed the working group wished to consider these issues. He said the working group document is not meant to be an FDA review. The subworking group rejected Dr. Miller's thirteenth comment.

Dr. Walters said Dr. Miller in his fourteenth comment said Section II-B is irrelevant to judging the appropriateness of a clinical trial.

Dr. Walters said the working group had included this section in the document in the hope of encouraging companies to take the patent route in protecting confidential information. This would permit greater public participation in review of human gene therapy proposals.

Mr. Mitchell said the key issue is whether the RAC and the working group would encourage public review of proposals.

The subworking group rejected Dr. Miller's fourteenth comment.

Dr. Walters said Dr. Miller in his fifteenth point expressed concern that the interrogatory style of the document may be interpreted as strident or even adversarial.

Dr. Gottesman did not think the document was perceived as strident or adversarial.

Dr. Anderson said there is no more adversarial organization than the FDA; he also found it extraordinary that Dr. Miller would attempt to give advice on how not to be adversarial.

[Executive Secretary's Note: The modified version of the points to consider is appended to these minutes as Attachment VII.]

Presentation to the RAC at the September 25, 1985, Meeting

Dr. Walters then asked the subworking group to suggest the optimal manner to present this document to RAC at the September 23, 1985, meeting. He said he would prefer that RAC formally accept this document and come to closure.

Dr. Gottesman suggested it might be useful to send to RAC a list of the modifications the subworking group introduced into the document at the September 20, 1985, meeting; this list would show none of the subworking group's modifications were substantive.

Dr. Anderson said the working group had received four comments on this version of the document: one set of comments was received from the working group's ad hoc advisor, Dr. Temin; the second set of comments was from Dr. Miller of the FDA; the third set of comments was from Mr. Lee Rogers on behalf of the Foundation on Economic Trends; and the fourth set of comments had been solicited from Dr. Myron Max Levine by Dr. Walters. The working group received only one set of comments from the general public--those of Mr. Rogers. In general, Mr. Rogers' comments were supportive.

Mr. Mitchell suggested Dr. Walters present to RAC a short history of the development of this document; this short history would then be part of the public record.

Dr. Gottesman asked Dr. Walters to remind RAC that the points to consider are a working document which will not be incorporated into the NIH Guidelines.

Mr. Mitchell asked Dr. Gartland how the document would be disseminated if accepted and adopted by RAC.

Dr. Gartland said the document would be published in the Recombinant DNA Technical Bulletin and sent to IRBs and IBCs.

Future Meeting Dates and Agendas

Dr. Walters asked the subworking group for suggestions on an appropriate date and appropriate agenda items for the next meeting of the Working Group on Human Gene Therapy. He suggested the working group in future meetings might: (1) continue a process of "self-education;" (2) discuss informed consent issues with representatives of OPRR and the NIH legal advisor; and (3) discuss germ line modification.

Dr. Gartland suggested the working group not focus specifically on germ line modification but discuss this issue in the context of other scientific issues.

Dr. Gottesman suggested the working group might consider meeting on the day following the winter/spring RAC meeting. RAC members who wish to attend could attend the working group meeting. Alternatively, some portion of the working group discussion could be scheduled as part of the RAC agenda. Certain topics, however, such as informed consent might be better discussed at a working group meeting.

Dr. Walters supported the suggestion to combine the working group and the RAC meetings; this would accentuate the ties between the RAC and the Working Group on Human Gene Therapy.

Dr. Anderson suggested another issue which should be discussed by the working group; i.e., when would it be appropriate for investigators to submit protocols to the working group for review. Some protocols may be sent to several different committees before being submitted for RAC review. He asked if investigators might send protocols to the working group before official submission for RAC review. He said important time would be lost if investigators must wait for IRBs and IBCs to complete review before the working group can begin review.

Dr. Gottesman thought the points to consider preclude RAC from reviewing the proposal before IBC and IRB review is complete. The points do not, however, preclude the working group from considering proposals before IRB and IBC review is complete. The working group would not, however, offer any recommendation before IRB and IBC approval is received.

Ms. Areen suggested the Introduction of the document might be modified to state:

> "Investigators are invited at their discretion to submit their proposal to the working group for simultaneous review during IRB and IBC review."

The subworking group agreed the language might read:

> "The principal investigator is invited to submit a copy of the protocol to the RAC and its working group at the time of submission to the IRB and the IBC."

Ms. Areen said protocols should not be treated as public documents until IRB and IBC review is completed. Dr. Murray agreed the IRB review process could be prejudiced by the release of information before completion of review.

Dr. Gartland said it would be very difficult under the present system to maintain confidentiality after submission of a proposal to the working group and RAC.

Dr. Walters suggested it was not appropriate to include any language on this topic in the points to consider without greater consideration of the ramifications. Dr. Gottesman agreed and pointed out that the points to consider as written do not forbid the working group from considering proposals before approval by IBCs and IRBs.

The subworking group agreed this issue should be considered in greater detail by the working group.

The meeting was adjourned at 12:45 p.m.

Respectfully submitted,

Elizabeth A. Milewski, Ph.D.
Rapporteur

William J. Gartland, Jr., Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

12/11/85
Date.

LeRoy Walters, Ph.D.
Chair

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ATTACHMENT II OF THE MINUTES OF THE SEPTEMBER 20, 1985, MEETING OF THE SUBWORKING GROUP ON HUMAN GENE THERAPY IS PAGES 33463 TO 33467 OF THE FEDERAL REGISTER OF AUGUST 19, 1985, WHICH CAN BE FOUND IN VOLUME 10 OF RECOMBINANT DNA RESEARCH.

ALBERT GORE, JR
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United States Senate

WASHINGTON, DC 20510

August 30, 1985

Hon. Margaret M. Heckler
Secretary
Department of Health & Human Services
200 Independence Ave., S.W.
Washington, D.C. 20201

Dear Secretary Heckler:

I am writing to express my concern over the current effort by the Food and Drug Administration and the proposed Biotechnology Science Board to usurp the role of the National Institutes of Health Recombinant DNA Advisory Committee in overseeing human gene therapy experiments. I am opposed to this effort, and I call upon you to end it immediately.

It is my understanding that plans are now being made within the Administration to make the RAC's authority to review proposals for gene therapy experiments secondary to that of the BSB itself. I am greatly disturbed by this development, for I do not believe that the BSB, as presently conceived, is a proper forum for the discussion of the questions raised by human gene therapy.

As you are well aware, the Biotechnology Science Board was proposed as the result of the increasing attention that is being given to the rapid development of biotechnology in this country and its implications. Particularly, the BSB resulted from concern over the potential consequences of the deliberate release of genetically engineered organisms into the environment and the role the federal government should play in regulating releases.

As Chairman of the House Science and Technology Subcommittee on Investigations and Oversight, I chaired the first congressional hearing on the deliberate release issue in June 1983. Subsequent to that hearing my subcommittee issued a report calling for the creation of an interagency group to review proposals for deliberate releases. The basis for that recommendation was the fact, underscored by witnesses at the hearing, that no single agency has both the needed expertise, experience, and authority to address the deliberate release problem adequately. This same conclusion was reached by other groups, including two other congressional committees, that later examined the issue.

The Biotechnology Science Board was proposed to correct this deficiency and to provide a mechanism for the evaluation of deliberate release proposals in a detailed and comprehensive fashion. The Board

is also designed to serve as a coordinating entity for biotechnology regulatory activities within the federal government. While the BSB will be involved in regulating aspects of commercial biotechnology, however, I believe that it is totally inappropriate for it to attempt to become involved at this time in oversight of human gene therapy experiments. The Board is simply not equipped for that purpose.

A vigorous public debate has taken place that supports the role of the Biotechnology Science Board in overseeing certain commercial biotechnology activities. Moroever, it is clear that the types of tasks to be performed by the BSB, such as the development of a workable risk assesssment methodology for deliberate release proposals, are best handled by an interagency group of this sort.

Similar conditions do not exist concerning BSB involvement in human gene therapy. The debate over gene therapy is just now beginning, and the issues of greatest importance are oriented more significantly toward ethical decisions, rather than purely scientific analysis. While obviously there are major scientific questions involved in any gene therapy experiment, the Recombinant DNA Advisory Committee has done an adequate job of addressing the scientific issues to date, and it appears capable of continuing to do so for the immediate future.

Both the Senate and House of Representatives have approved legislation to establish a national commission on bioethics, and it is anticipated that this legislation will become law before the end of this year. The first issue to be considered by the commission will be human genetic engineering. The commission will consider the prospects for human gene therapy in this country and its implications and will report its findings to the Congress. The commission will undoubtedly consider whether a formalized regulatory structure, like the BSB, for gene therapy will be needed. Until the appropriateness of any such regulatory regime is clear, however, the BSB should abstain from any effort to bring gene therapy within its purview.

It is my desire to see the Biotechnology Science Board proceed with the mission for which it was created. Attempts to expand the Board into areas for which it is ill-equipped can only divert it from that mission and result in self-defeating controversy.

I look forward to your reply.

Sincerely,

Albert Gore Jr.
United States Senator

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1244

September 18, 1985

BY MESSENGER

Director, Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, Maryland 20205

Comments of the Foundation on Economic Trends and Jeremy Rifkin on the NIH Notice issued August 19, 1985, 50 Fed. Reg. 33462, re IV. Points To Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols.

In the Introduction (3), it is stated that the Points to Consider document "will be considered for revision at least annually". There may well be a need for frequent revisions as experience is gained in this field. There should be no a priori assumption regarding how soon or often revisions may be necessary. A particular experiment(s) may raise issues requiring a proposed revision within a few months rather than annually.

In the Introduction (11), the potential of developments that "enhance human capabilities rather than to correct defects in patients" is recognized as possible, albeit not as immediate or inevitable, results of such research. Accordingly, the working group states that it "will cooperate with other groups in assessing the possible long-term consequences of somatic-cell gene therapy and related . . . experiments . . . to define appropriate human applications of this emerging technology." Inasmuch as rapid developments in this field seem inevitable, we recommend that the working group begin immediately to develop the procedural structure for such cooperative efforts and to review its own composition to determine if it should be broadened or if its balance of disciplines should be altered.

The working groups speaks of cooperating with other groups

without defining who or what they may be. The procedural structure should assure meaningful participation by interested members of the public, including representatives of groups, before final recommendations on any particular proposal are made.

A given proposal may raise value-laden social and ethical issues that require more than the application of technical or scientific disciplines by the working group as well as by those groups that propose specific studies. There may be need for qualified representatives of the social sciences, including theologians, ethicists, social philosophers, anthropoligists, and economists, as well as micro-biologists and various medical experts. The existence of only one or two representatives of such other disciplines may not be sufficient to recognize the potential issues or to resolve them.

In Part I, B, 5, "Qualification of Investigators" etc., it is requested that the "relevant training and experience of the personnel" be disclosed (with a cross-reference in paragraph a to Section III-D, which should have been to Section III-E). Reference is made both to "medical and nonmedical" professional personnel. We suggest that the document be amended to list those disciplines that the Working Group considers may be relevant in the development of such studies and proposals.

In keeping with its limited function, much of the Points to Consider is simply a listing of relevant questions with virtually no guidance at this time as to what would or would not be acceptable to the working group or to the RAC in terms of potential risks and offsetting benefits involved, or of value issues raised. We refer particularly to most of the questions asked in Part I. We recommend that as promptly as possible, as experience is gained, the working group begin the more difficult task of addressing such risk-benefit and value-resolution issues questions utilizing the inter-disciplinary approach appropriate for such an endeavor. This task should not be deferred until the RAC and the NIH are faced immediately with proposals with long-term consequences as discussed in the Introduction (11) and referred to above.

The working group should develop criteria for Part II (Special Issues) immediately, rather than simply ask the questions there posed. Procedures should be proposed now that applicants would be required to follow to assure accurate information to the public on issues of

concern to them, and to assure as full communication as possible to the concerned professionals of research methods and results.

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August 29, 1985

Dr. William Gartland
Director, Office of Recombinant
DNA Activities
NIAID, NIH
Bldg. 31, Room 4A52
Bethesda, MD 20205

Dear Bill:

I have read the proposed outline on guidelines for human somatic gene therapy. I consider it excellent, balanced, conservative, and sensitive to social issues. My only notable comment relates to item 5 a-d on p.18. I believe that the points listed therein should be prerequisites for patients to participate in the earliest clinical protocols. In this way the highest ethical standards will be followed maximum, while a maximum of scientific information is obtained that can be applied toward modification of future protocols.

Sincerely,

Myron M. Levine, M.D., D.T.P.H.
Professor and Director
Center for Vaccine Development

MML/rr

5. Will patients and/or their parents or guardians be informed:

a. That some of the procedures performed in the study will be irreversible.

b. That following the performance of such procedures it would not be medically advisable for patients to withdraw from the study?

c. That a willingness to cooperate in long-term follow-up (for at least three to five years) will be a precondition for participation in the study?

d. That a willingness to permit an autopsy to be performed in the event of a patient's death following treatment is also a precondition for a patient's participation in the study? (This stipulation is included because an accurate determination of the precise cause of a patient's death would be of vital importance to all future gene therapy patients.)

E. Privacy and confidentiality

Indicate what measures will be taken to protect the privacy of gene therapy patients and their families as well as to maintain the confidentiality of research data.

1. What provisions will be made to honor the wishes of individual patients (and the parents or guardians of pediatric or mentally handicapped patients) as to whether, when, or how the identity of patients is publicly disclosed?

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

Food and Drug Administration
Rockville MD 20857

SEP 13 [illegible]

1243

Dr. William J. Gartland, Jr.
Director, Office of Recombinant DNA Activities
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NIH
Bethesda, MD 20205

Dear Bill,

The FDA appreciates the efforts of the RAC's Working Group on Human Gene Therapy to draft a document that will provide guidance to clinical investigators about to embark on somatic-cell gene therapy. As you are aware, the FDA has provided similar documents on a variety of subjects and these have, we are certain, expedited the submission and review process.

My colleagues and I have reviewed carefully the current draft of the NIH's "Points to Consider," and on the following pages we offer some general and specific comments regarding the document. We believe that the document will require some revision prior to its promulgation in final form and hope that our comments will be helpful. As always, through our liaison representatives, we look forward to continued collaboration between NIH and FDA in this area.

Sincerely yours,

Henry I. Miller, MD
Food & Drug Administration Liaison
to the RAC

FDA'S COMMENTS ON NIH'S PROPOSED "POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF HUMAN SOMATIC-CELL GENE THERAPY PROTOCOLS"

1. In the discussion of proprietary information in gene therapy proposals, it should be noted that where proprietary information is included, those portions of proposals would be discussed during closed sessions. While this may not occur frequently, the option for closed sessions should be available.

2. Paragraph (10) focuses very narrowly on "undesirable consequences of somatic-cell gene therapy," and is misleading. First, it ignores that the appropriateness of medical therapies is the result of complex risk/benefit judgements, and implies that any risk of teratogenesis ("vertical transmission of genetic changes from an individual to his or her offspring") is unacceptable. As evidenced by the use in fertile patients of radiotherapy, chemotherapy, and many drugs believed to have some teratogenic potential, anticipated benefits may outweigh possible risks.

3. In section I.B.1.b., the wording in the first sentence would be more clear if it were changed to, "What is the composition of the material . . ."

4. In section I.B.1.b., we suggest the addition of two new subsections:

    a. One would request the investigator to, "Describe in detail the methods for harvesting, extraction, and purification, and for the removal of any toxic chemicals introduced by these procedures."

    b. The second would contain a warning that penicillin and other beta-lactam-containing antibiotics should not be used in the production of materials administered to patients.

5. In section I.B.1.b.(1), we suggest the addition of questions about: (1) the methods for assaying potency of the product used to treat cells or administered to a patient; and (2) the consistency of the product lot-by-lot, if different lots will be used on the same patient or on different patients; and (3) the stability of the product under the projected conditions of storage.

6. In section I.B.2.c., we suggest the addition of a section that would request a description of previously-reported similar human studies (including foreign studies) and their results.

7. Section I.B.2.c.(1)(c) is relevant to gene therapy whether or not a retroviral system is used, and should therefore be relocated just prior to I.B.2.c.(1).

8. In section I.B.2.c.(1)(d), we believe that the phrase "specifically germline cells" should be deleted because apprehension about an effect on gametes should be not greater than for other critical tissues including, for example, blood or bone cell precursors. A clearer, more appropriate way of posing the question would be, "What is the specificity of transformation or other effects for the target cells?"

9. In section I.B.2.c.(1)(e), the first sentence would be more clear if revised to read, "Describe animal experiments completed or in progress employing protocols similar to that proposed."

10. We believe that section I.B.3. is the appropriate place for inclusion of "selection of subjects," rather than as a separate section I.C.

11. In section I.B.3.e., we suggest that the first sentence of the section is sufficient. The remainder requires of the investigator extremely complex speculation and should be deleted.

12. In section I.D., the initial paragraph under "informed consent" would provide sufficient guidance, without points 1-5, if the following were added to the paragraph:

> "The consent form must adhere to the requirements of 45 CFR 46 and 21 CFR 50. Special attention must be paid to the applicability of the additional elements of informed consent listed in these regulation. (Include a copy of the patient consent form as part of the documentation requested in Part III, below.)"

Points 1-5 are a partial listing of the elements of informed consent and may be misleading to applicants. If a listing of details of elements of informed consent is deemed necessary, we believe it shhould include all of the basic and additional elements of informed consent contained in the regulations.

13. In section I.E., the initial paragraph under "Privacy and Confidentiality" would, we believe, provide sufficient information, without points 1 and 2.

14. We believe that section II.B. is irrelevant to a judgement of the appropriateness of a clinical trial.

15. We are concerned that the interrogatory style of the document may be interpreted as strident or even adversary. Furthermore, the document does not provide the rationale for many of the questions although arguably the rationale could be invaluable for the formulation of responses. We believe that both the tone of the document and the quality of communication between NIH and investigators would be improved by a more explicative, collaborative approach. As a n example of this, Attachment A is the FDA's draft "Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology."

16. We have one additional major concern. There exists virtual unanimity among those conversant with human gene therapy that somatic-cell gene therapy is analogous to other conventional medical interventions, such as vaccination, exogenous replacement of insulin in diabetics, or heterologous organ transplantation for genetic disease (see, for example, Barranger, New Eng. J. Med. 311, 1629-30, 1984). The conclusion of the recent OTA report, Human Gene Therapy-Background Paper, which is in fact cited in the "Points" document, is instructive:

> Civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies.

The FDA has also endorsed this view in principle and has made known its intention to subject appropriate products employed for human gene therapy to an adminstrative review similar to that for analogous biological drug products. Since as implied above, most of the critical decisions on individual

investigators' submissions are likely to be scientific and medical ones, we suggest reconsideration of the membership of the RAC Working Group on Human Gene Therapy [which will conduct the initial administrative review of proposals] which currently includes a large proportion of attorneys, ethicists, and public policy specialists. Arguably, the Working Group should be supplemented with more members with expertise in molecular biology, pharmacology, medicinal chemistry, and medicine.

0011A

NATIONAL INSTITUTES OF HEALTH
POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF
HUMAN SOMATIC-CELL GENE THERAPY PROTOCOLS

WORKING GROUP ON HUMAN GENE THERAPY
NIH RECOMBINANT DNA ADVISORY COMMITTEE

OUTLINE

Applicability

Introduction

I. Description of Proposal

A. Objectives and rationale of the proposed research

B. Research design, anticipated risks and benefits

1. Structure and characteristics of the biological system

2. Preclinical studies, including risk assessment studies

3. Clinical procedures, including patient monitoring

4. Public-health considerations

5. Qualifications of investigators, adequacy of laboratory and clinical facilities

C. Selection of patients

D. Informed consent

E. Privacy and confidentiality

II. Special Issues

A. Provision of accurate information to the public

B. Timely communication of research methods and results to investigators and clinicians

III. Requested Documentation

A. Original protocol

B. IRB and IBC minutes and recommendations

C. One-page abstract of gene therapy protocol

D. One-page description of proposed experiment in non-technical language

E. Curricula vitae for professional personnel

F. Indication of other federal agencies to which the protocol is being submitted

G. Other pertinent material

IV. Reporting Requirements

NATIONAL INSTITUTES OF HEALTH

POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF HUMAN

SOMATIC-CELL GENE THERAPY PROTOCOLS

Applicability - These "Points to Consider" apply only to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from the National Institutes of Health (NIH). This includes research performed by NIH directly.

Introduction

(1) Experiments in which recombinant DNA[1] is introduced into cells of a human subject with the intent of stably modifying the subject's genome are covered by Section III-A-4 of the NIH Guidelines for Research Involving Recombinant DNA Molecules (49 Federal Register 46266). Section III-A-4 requires such experiments to be reviewed by the NIH Recombinant DNA Advisory Committee (RAC) and approved by the NIH. RAC consideration of each proposal will be on a case-by-case basis and will follow publication of a precis of the proposal in the Federal Register, an opportunity for public comment, and a review of the proposal by the working group of the RAC. RAC recommendations on each proposal will be forwarded to the NIH Director for a decision which will then be published in the Federal Register. In accordance with Section IV-C-1-b of the NIH Guidelines, the NIH Director may approve proposals only if he finds that they present "no significant risk to health or the environment."

(2) In general, it is expected that somatic-cell gene therapy protocols will not present a risk to the environment as the recombinant DNA is expected to be confined to the human subject. Nevertheless, Section I-B-4-b of the "Points to Consider" document asks the researchers to address specifically this point.

[1]Section III-A-4 applies both to recombinant DNA as well as to DNA derived from recombinant DNA.

(3) This document is intended to provide guidance in preparing proposals for NIH consideration under Section III-A-4 of the NIH Guidelines for Research Involving Recombinant DNA Molecules. Not every point mentioned in the "Points to Consider" document will necessarily require attention in every proposal. The document will be considered for revision as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out at least annually.

(4) A proposal will be considered by the RAC only after the protocol has been approved by the local Institutional Biosafety Committee (IBC) and by the local Institutional Review Board (IRB) in accordance with Department of Health and Human Services (DHHS) Regulations for the Protection of Human Subjects (45 Code of Federal Regulations, Part 46). If a proposal involves children, special attention should be paid to subpart D of these DHHS regulations. The IRB and IBC may, at their discretion, condition their approval on further specific deliberation by the RAC and its working group. Consideration of gene therapy proposals by the RAC may proceed simultaneously with review by any other involved federal agencies[2] provided that the RAC is notified of the simultaneous review. Meetings of the committee will be open to the public except where trade secrets or proprietary information would be disclosed. The committee would prefer that the first proposals submitted for RAC review contain no proprietary information or trade secrets, enabling all aspects of the review to be open to the public. The public review of these protocols will serve to inform the

[2]The Food and Drug Administration (FDA) has jurisdiction over drug products intended for use in clinical trials of human somatic-cell gene therapy. For general information on FDA's policies and regulatory requirements, please see the Federal Register, Volume 49, pages 50878-80, 1984.

public not only on the technical aspects of the proposals but also on the meaning and significance of the research.

(5) The clinical application of recombinant DNA techniques to human gene therapy raises two general kinds of questions: (1) the questions usually discussed by IRBs in their review of any proposed research involving human subjects; and (2) broader social issues. The first type of question is addressed principally in Part I of this document. Several of the broader social issues surrounding human gene therapy are discussed later in this Introduction and in Part II below.

(6) Following the Introduction, this document is divided into four parts. Part I deals with the short-term risks and benefits of the proposed research to the patient[3] and to other people, as well as with issues of fairness in the selection of patients, informed consent, and privacy and confidentiality. In Part II, investigators are requested to address special issues pertaining to the free flow of information about clinical trials of gene therapy. These issues lie outside the usual purview of IRBs and reflect general public concerns about biomedical research. Part III summarizes other requested documentation that will assist the RAC and its working group in their review of gene therapy proposals. Part IV specifies reporting requirements.

(7) A distinction should be drawn between making genetic changes in somatic cells and in germ line cells. The purpose of somatic cell gene therapy is to treat an individual patient, e.g., by inserting a properly functioning

[3]The term "patient" and its variants are used in the text as a shorthand designation for "patient-subject."

gene into a patient's bone marrow cells in vitro and then reintroducing the cells into the patient's body. In germ line alterations, a specific attempt is made to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring. The RAC and its working group will not at present entertain proposals for germ line alterations but will consider for approval protocols involving somatic-cell gene therapy.

(8) The acceptability of human somatic-cell gene therapy has been addressed in several recent public documents as well as in numerous academic studies. The November 1982 report of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, Splicing Life, resulted from a two-year process of public deliberations and hearings; upon release of that report, a House subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, Human Gene Therapy, which brought these earlier documents up-to-date. As the latter report concluded:

> "Civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies."

(9) Concurring with this judgment, the RAC and its working group are prepared to consider for approval somatic-cell therapy protocols, provided that the design of such experiments offers adequate assurance that their consequences will not go beyond their purpose, which is the same as the traditional purpose of all clinical investigations, namely, to benefit the health and well-being of the individual being treated while at the same time gathering generalizable knowledge.

(10) Two possible undesirable consequences of somatic-cell therapy would be unintentional (1) vertical transmission of genetic changes from an individual to his or her offspring or (2) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, this document requests information that will enable the RAC and its working group to assess the likelihood that the proposed somatic-cell gene therapy will inadvertently affect reproductive cells or lead to infection of other people (e.g., treatment personnel or relatives).

(11) In recognition of the social concern that surrounds the general discussion of human gene therapy, the working group will continue to consider the possible long-range effects of applying knowledge gained from these and related experiments. While research in molecular biology could lead to the development of techniques for germ line intervention or for the use of genetic means to enhance human capabilities rather than to correct defects in patients, the working group does not believe that these effects will follow immediately or inevitably from experiments with somatic-cell gene therapy. The working group will cooperate with other groups in assessing the possible long-term consequences of somatic-cell gene therapy

and related laboratory and animal experiments in order to define appropriate human applications of this emerging technology.

(12) Responses to the questions raised in these "Points to Consider" should be provided in the form of either written answers or references to specific sections of the protocol or its appendices.

I. Description of Proposal

A. Objectives and rationale of the proposed research

State concisely the overall objectives and rationale of the proposed study. Please provide information on the following specific points:

1. Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?

2. Describe the natural history and range of expression of the disease selected for treatment. In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene therapy?

3. Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

4. What alternative therapies exist? In what groups of patients are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene therapy?

B. Research design, anticipated risks and benefits

1. Structure and characteristics of the biological system

Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

a. What is the structure of the cloned DNA that will be used?

(1) Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.

(2) What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)?

(3) Describe the steps used to derive the DNA construct.

b. What is the structure of the material that will be administered to the patient?

(1) Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells.

(a) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)?

What tests have been used and what is the sensitivity of the tests?

(b) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (nucleic acids, proteins, etc.) or contaminating viruses or other organisms in the cells or serum used for preparation of the virus stock?

(2) Describe any other material to be used in preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

2. Preclinical studies, including risk-assessment studies

Describe the experimental basis (derived from tests in cultured cells and animals) for claims about the efficacy and safety of the proposed system for gene delivery.

a. Laboratory studies of the delivery system

(1) What cells are the intended recipients of gene therapy? If recipient cells are to be treated in vitro and returned to the patient, how will the cells be characterized before

and after treatment? What is the theoretical and practical basis for assuming that only the treated cells will act as recipients?

(2) Is the delivery system efficient? What percentage of the target cells contain the added DNA?

(3) How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

(4) How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?

b. Laboratory studies of gene expression

Is the added gene expressed? To what extent is expression only from the desired gene (and not from the surrounding DNA)? In what percentage of cells does expression from added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene? Is the gene expressed in cells other than the target cells? If so, to what extent?

c. Laboratory studies pertaining to the safety of the delivery/expression system

(1) If a retroviral system is used:

(a) What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

(b) How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

(c) What laboratory evidence is available concerning potential harmful effects of the treatment, e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses? What steps have been taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

(d) Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ line cells? What is the sensitivity of the analyses?

(e) Has a protocol similar to the one proposed for a clinical trial been carried out in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

(2) If a non-retroviral delivery system is used: What animal studies have been done to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ line cells)? How long have the animals been studied after treatment? What tests have been used and what is their sensitivity?

3. Clinical procedures, including patient monitoring

Describe the treatment that will be administered to patients and the diagnostic methods that will be used to monitor the success or failure of the treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study.

a. Will cells (e.g., bone marrow cells) be removed from patients and treated in vitro in preparation for gene therapy? If so, what kinds of cells will be removed from the patients, how many, how often, and at what intervals?

b. Will patients be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy) prior to gene therapy?

c. What treated cells (or vector/DNA combination) will be given to patients in the attempt to administer gene therapy? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple treatments? If so, over what period of time?

d. What are the clinical endpoints of the study? How will patients be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be done? How long will patient follow-up continue?

e. What are the major potential beneficial and adverse effects of treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of potential adverse effects on patients with the probability and magnitude of deleterious consequences from the disease if gene therapy is not performed.

f. If a treated patient dies, what special studies will be performed as part of the autopsy?

4. Public-health considerations

Describe any potential benefits and hazards of the proposed therapy to persons other than the patients being treated. Specifically:

a. On what basis are potential public health benefits or hazards postulated?

b. Is there a significant likelihood that the added DNA will spread from the patient to other persons or to the environment?

c. Will any precautions be taken against such spread (e.g., to patients sharing a room, health-care workers, or family members)?

d. What measures will be undertaken to mitigate the risks, if any, to public health?

5. Qualifications of investigators, adequacy of laboratory and clinical facilities

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of gene therapy. In addition, please describe the laboratory and clinical facilities where the proposed study will be performed.

a. What professional personnel (medical and nonmedical) will be involved in the proposed study? What are their specific qualifications and experience with respect to the disease to be treated and with respect to the techniques employed in molecular biology? Please provide curricula vitae (see Section III-E).

b. At what hospital or clinic will the treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will patients occupy regular hospital beds or clinical research center beds? Where will patients reside during the follow-up period?

C. Selection of patients

Estimate the number of patients to be involved in the proposed study of gene therapy. Describe recruitment procedures and patient eligibility requirements, paying particular attention to whether these procedures and requirements are fair and equitable.

1. How many patients do you plan to involve in the proposed study?

2. How many eligible patients do you anticipate being able to identify each year?

3. What recruitment procedures do you plan to use?

4. What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

5. How will patients be selected if it is not possible to include all who desire to participate?

D. Informed consent

Indicate how patients will be informed about the proposed study and how their consent will be solicited. The consent procedure should adhere to the requirements of DHHS regulations for the protection of human subjects (45 Code of Federal Regulations, Part 46). If the study

involves pediatric or mentally handicapped patients, describe procedures for seeking the permission of parents or guardians and, where applicable, the assent of each patient. Areas of special concern highlighted below include potential adverse effects, financial costs, privacy, and long-term follow-up.

1. How will the major points covered in Sections I-A through I-C of this document be disclosed to potential participants in this study and/or parents or guardians in language that is understandable to them?

2. How will the innovative character and the theoretically-possible adverse effects of gene therapy be discussed with patients and/or parents or guardians? How will the potential adverse effects be compared with the consequences of the disease? What will be said to convey that some of these adverse effects, if they occur, could be irreversible?

3. What explanation of the financial costs of gene therapy and any available alternative therapies will be provided to patients and/or parents or guardians?

4. How will patients and/or their parents or guardians be informed that the innovative character of gene therapy may lead to great interest by the media in the research and in treated patients?

5. How will patients and/or their parents or guardians be informed:

   a. That some of the procedures performed in the study may be irreversible?

   b. That following the performance of such procedures it would not be medically advisable for patients to withdraw from the study?

   c. That a willingness to cooperate in long-term follow-up (for at least three to five years) will be a precondition for participation in the study?

   d. That a willingness to permit an autopsy to be performed in the event of a patient's death following treatment is also a precondition for a patient's participation in the study? (This stipulation is included because an accurate determination of the precise cause of a patient's death would be of vital importance to all future gene therapy patients.)

E. Privacy and confidentiality

Indicate what measures will be taken to protect the privacy of gene therapy patients and their families as well as to maintain the confidentiality of research data.

1. What provisions will be made to honor the wishes of individual patients (and the parents or guardians of pediatric or mentally handicapped patients) as to whether, when, or how the identity of patients is publicly disclosed?

2. What provision will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual patients?

II. Special Issues

Although the following issues are beyond the normal purview of local IRBs, the RAC and its working group request that investigators respond to questions A and B below.

A. What steps will be taken, consistent with point I-E above, to ensure that accurate information is made available to the public with respect to such public concerns as may arise from the proposed study?

B. Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among investigators and clinicians concerning research methods and results?

III. Requested Documentation

In addition to responses to the questions raised in these "Points to Consider," please submit the following materials:

A. Your protocol as approved by your local IRB and IBC. The consent form, which must have IRB approval, should be submitted to the NIH only on request.

B. Local IRB and IBC minutes and recommendations that pertain to your protocol.

C. A one-page scientific abstract of the gene therapy protocol.

D. A one-page description of the proposed experiment in nontechnical language.

E. Curricula vitae for professional personnel.

F. An indication of other federal agencies to which the protocol is being submitted for review.

G. Any other material which you believe will aid in the review.

IV. Reporting Requirements

A. Serious adverse effects of treatment should be reported immediately to both your local IRB and the NIH Office for Protection from Research Risks, and a written report should be filed with both groups. A copy of the report should also be forwarded to the NIH Office of Recombinant DNA Activities (ORDA).

B. Reports regarding the general progress of patients should be filed at six-month intervals with both your local IRB and ORDA. These twice-yearly reports should continue for a sufficient period of time to allow observation of all major effects (at least three to five years). In the event of a patient's death, the autopsy report should be submitted to the IRB and ORDA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING

SEPTEMBER 23, 1985

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
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RECOMBINANT DNA ADVISORY COMMITTEE

MINUTES OF MEETING[1]

SEPTEMBER 23, 1985

The Recombinant DNA Advisory Committee (RAC) was convened for its thirty-third meeting at 9:00 a.m. on September 23, 1985, in Building 31, Conference Room 6, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892. Mr. Robert Mitchell (Chair), Attorney at Law in California, presided. In accordance with Public Law 92-463, the meeting was open to the public. The following were present for all or part of the meeting:

Committee members:

Barbara Bowman
Royston Clowes
Mitchell Cohen
L. Albert Daloz
Bernard Davis
Susan Gottesman
Irving Johnson
Arthur Landy
David Martin
John McGonigle
Mark Mills
Robert Mitchell
Thomas Pirone
David Pramer
Fred Rapp
LeRoy Walters
Pieter Wensink
Anne Witherby
William J. Gartland, Jr. (Executive Secretary)

A committee roster is attached (Attachment I).

Ad hoc consultants:

John Collier, Harvard Medical School
Anthony MacQuillan, University of Maryland
Robert Mortimer, University of California, Berkeley
Thomas Waldmann, National Institutes of Health

[1]The RAC is advisory to the NIH, and its recommendations should not be considered as final or accepted. The Office of Recombinant DNA Activities should be consulted for NIH policy on specific issues.

TABLE OF CONTENTS

Part		Page
I.	Call to Order and Opening Remarks	5
II.	Minutes of the May 3, 1985, RAC Meeting	5
III.	Report of Working Group on Human Gene Therapy	5
IV.	Biotechnology Science Board	13
V.	Proposed Amendments of Appendix C-III	18
VI.	Proposed Amendment of Part III of the NIH Guidelines	20
VII.	Request to Clone Shiga-like Toxin from Enterobacteriaceae and Vibrionaceae	21
VIII.	Request to Clone a Hybrid Toxin Gene	24
IX.	Proposed Exchanger List for Gram Positive Bacteria	28
X.	Future Meeting Dates	28
XI.	Other Matters	28
XII.	Adjournment	29

Non-voting agency representatives:

John R. Fowle, Environmental Protection Agency
Bernard Greifer, Department of Commerce
Bernadine Healy, Office of Science and Technology Policy, Executive Office of the President
Morris A. Levin, Environmental Protection Agency
Audrey Manley, Health Resources and Services Administration
Edwin Shykind, Department of Commerce
Sue A. Tolin, Department of Agriculture
William J. Walsh, Department of State

National Institutes of Health staff:

W. French Anderson, NHLBI
Stanley Barban, NIAID
Becky Connors, NIAID
Rosalind Gray, OD
Hortencia Hornbeak, NIAID
Rachel Levinson, OD
Elizabeth Milewski, NIAID
Virginia Ono, OD
Donald Ralbovsky, OD
Bernard Talbot, NIAID

Others:

Joan C. Amatniek, The Chronicle of Higher Education
Irene Brandt, Eli Lilly and Company
Alice J. Caddow, Genencor, Inc.
Patricia Campbell, Uniformed Services University of the Health Sciences
Louise Cannon, StenoTech, Inc.
Chia Ting Chen, Department of Labor
Joel Dalrymple, Department of Defense
Mary Ann Danello, Food and Drug Administration
Marie A. Dray, Pharmaceutical Manufacturers Association
Charles J. Eby, Monsanto Company
Peter Farnham, American Society for Biological Chemists
Jeffrey Fox, American Society for Microbiology
Robert J. Frederick, Environmental Protection Agency
Mary Gant, Office of Science and Technology Policy, Executive Office of the President
David Glass, BioTechnica International, Inc.
Alan Goldhammer, Industrial Biotechnology Association
Carol Lax Gronbeck, Genentech, Inc.
Stuart Harris, Beach Media
Dorothy Jessup, Department of Agriculture
Roger S. Johnson, Genetic Engineering News

Attila T. Kadar, Food and Drug Adminstration
John H. Keene, Abbott Laboratories
Gail Kidder, Beach Media
Rihito Kimura, Kennedy Institute
Lisa Krieger, American Medical News
Myron M. Levine, University of Maryland, Baltimore
Kathryn R. Mahaffey, National Institute for Occupational Safety and Health
Linda Mayhew, Beach Media
Douglas K. McCormick, Bio/Technology
Julie Miller, Science News
Lorraine C. Minecci, Wyeth Laboratories
John Murphy, University Hospital at Boston University Medical Center
Kerry Myers, Beach Media
Alison O'Brien, Uniformed Services University of the Health Sciences
Steve Olson, National Academy of Sciences
Greg Pearson, Blue Sheet
Julie Presar, Foundation on Economic Trends
Harvey S. Price, Industrial Biotechnology Association
Rex Rhein, McGraw-Hill World News
Jeremy Rifkin, Foundation on Economic Trends
Marvin Rogul, The Rogul Group
T. Sakata, Embassy of Japan
Harold M. Schmeck, New York Times
Mark C. Segal, Environmental Protection Agency
George Shibley, Department of Agriculture
Smita K. Siddhanti, University of Pittsburgh
Arthur Spring, Commerce Clearing House
Paul E. Stern, University of Florida
Ashley Stevens, BioTechnica International, Inc.
Clarence E. Styron, Monsanto Company
Marjorie Sun, Science Magazine
Laura Tangley, Bioscience
Marshall Tarkington, Department of Agriculture
Joseph Van Houten, Schering-Plough
Marian Willinger, Harvard Medical School
Roger Yocum, BioTechnica International, Inc.

(Note: Page 4 is a blank page when the minutes are published in booklet form.)

I. CALL TO ORDER AND OPENING REMARKS

Mr. Mitchell, Chair, called the September 23, 1985, meeting of the Recombinant DNA Advisory Committee (RAC) to order. Mr. Mitchell said formal notice of this public meeting had been given in the August 19, 1985, Federal Register (50 FR 33462).

Dr. Gartland informed Mr. Mitchell a quorum was present at the meeting. He announced to RAC that Mr. Mitchell had been reappointed Chair of the RAC through June 1987.

Mr. Mitchell said he would recognize speakers in the following order: primary reviewers; other RAC members; ad hoc consultants; non-voting representatives to the RAC; RAC's administrative staff; members of the public who submitted written documents or comments; and finally other members of the public who wish to comment.

Mr. Mitchell introduced the ad hoc consultants to this meeting of the RAC: Dr. John R. Collier of Harvard Medical School; Dr. Robert K. Mortimer of the University of California, Berkeley; Dr. Anthony MacQuillan of the University of Maryland; and Dr. Thomas A. Waldmann of the National Cancer Institute of the National Institutes of Health (NIH).

II. MINUTES OF THE MAY 3, 1985, RAC MEETING

Mr. Daloz said he had reviewed the minutes (tab 1234) of the May 3, 1985, RAC meeting. He called attention to Dr. Gottesman's remarks concerning education of the public in the ethical, moral, and legal issues surrounding recombinant DNA.

Dr. Mills said he had also reviewed the minutes of the May 3, 1985, meeting and to the best of his knowledge the minutes are correct.

Dr. Johnson moved acceptance of the minutes. Dr. Gottesman seconded the motion. By a vote of seventeen in favor, none opposed, and no abstentions the minutes were accepted.

III. REPORT OF WORKING GROUP ON HUMAN GENE THERAPY

Mr. Mitchell asked Dr. Walters, the Chair of the RAC Working Group on Human Gene Therapy, to report on the progress of this working group's attempt to develop a guidance document (tabs 1232, 1235/IV, 1243, 1244, 1245, 1246) for investigators submitting proposals involving human gene therapy.

Dr. Walters reported that the Working Group on Human Gene Therapy published an initial version of the "Points to Consider in the Design and Submission

of Human Somatic-Cell Gene Therapy Protocols" in the January 22, 1985, Federal Register. Fourteen letters commenting on the points to consider were received by the NIH in response to this announcement.

These comments were carefully reviewed by the working group at its April 1, 1985, meeting; and the changes agreed upon by the working group were incorporated into the April 15, 1985, version of the document. That version of the document was considered by RAC at its May 3, 1985, meeting; and modifications were introduced into the document on the basis of that discussion.

Dr. Walters then summarized the differences between the April 15 version and the subsequent version published in the August 19, 1985, Federal Register: (1) A section entitled "Applicability" was added to the document. This section explicitly states that the document applies to projects conducted at or sponsored by an institution that receives NIH support for recombinant DNA research. (2) A footnote, which in the January 22 version referred to both recombinant DNA and recombinant RNA, was changed to refer only to recombinant DNA, since the current NIH Guidelines only explicitly cover recombinant DNA and not recombinant RNA. (3) A footnote indicating the Food and Drug Administration (FDA) has jurisdiction over drug products intended for use in clinical trials of human somatic-cell gene therapy was added to the document. (4) A request for submission to NIH of the informed consent form was deleted from the document. In general, the NIH does not review consent forms; local Institutional Review Boards (IRBs) review and approve consent forms. However, language indicating NIH may request consent forms for human gene therapy protocols in certain circumstances was added to Section III-A of the points to consider document.

Dr. Walters said three comment letters were received on the revised version of the document published in the Federal Register of August 19, 1985. In addition, a working group consultant, Dr. Howard Temin of the University of Wisconsin, commented by telephone.

A Subworking Group on Human Gene Therapy met on September 20, 1985, to address these comments. In response to these comments, the subworking group introduced fourteen minor technical amendments into the document.

Dr. Walters enumerated these modifications. First, the subworking group highlighted its intention to revise the document as developments dictate by modifying the last sentence of paragraph (3) of the Introduction as follows:

> "The document will be considered for revision as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out at least annually."

Dr. Walters said the second minor technical change appears in paragraph (10) of the Introduction; the subworking group deleted the article "The" preceding "two possible undesirable consequences" of somatic-cell therapy.

Dr. Walters said the third minor modification occurs in Section I-B-1-a-(1); additional descriptive adjectives were added to the second sentence in the section. The subworking group modified that section to read:

> "Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct."

Dr. Walters said language was added to Section I-B-1-b-(1)-(b); the last sentence of that section now reads as follows:

> "What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (nucleic acids, proteins, etc.) or contaminating viruses or other organisms in the cells or serum used for preparation of the virus stock?"

Dr. Walters said the fifth change was made in Section I-B-2; the subworking group deleted the word "laboratory" since the phrase "laboratory animals" suggests mice and rats to many investigators.

Dr. Walters said Dr. Temin had suggested the word "added" be substituted for the word "inserted" in Section I-B-2-a-(2), Section I-B-2-a-(4), Section I-B-2-b, and Section I-B-4-b of the document since DNA might be added to cells but not inserted in the chromosome. The subworking group accepted this suggestion for Section I-B-2-a-(2), Section I-B-4-b, the second sentence of Section I-B-2-a-(4), and the first sentence of Section I-B-2-b.

Dr. Walters said the subworking group had also modified the third sentence of Section I-B-2-b to clearly state its interest in expression of the "added" DNA.

Dr. Walters said a typographical error in Section I-B-5-a was corrected; that section should refer to Section III-E rather than to Section III-D.

Dr. Walters said Section I-D-4 has been modified to inquire "how" patients or guardians will be informed that the innovative character of gene therapy may stimulate great media interest in the procedures and subjects. A second question referring to the protection of patients' privacy was deleted from this section because that topic is adequately covered in Section I-E.

Dr. Walters said Section I-D-5 has also been modified to inquire "how" patients and/or their guardians will be informed that some procedures may be irreversible, that it may not be medically advisable to withdraw from the study, that patients will be required to participate in long-term follow-up, and that an autopsy would be required.

Dr. Walters said the fourteenth modification was introduced in Section II-A in recognition of the tension between providing the greatest possible information to the public while protecting the privacy of patients and families. Section II-A has been modified to read as follows:

> "What steps will be taken, consistent with point I-E above, to ensure that accurate information is made available to the public with respect to such public concerns as may arise from the proposed study?"

Dr. Walters said Mr. Edward Lee Rogers for the Foundation on Economic Trends had commented (tab 1244) that the working group should begin immediately to develop cooperative efforts with other groups to evaluate long-term implications of human gene therapy. Dr. Walters said working group members have already collaborated with the Office of Technology Assessment in the preparation of the 1984 background paper, Human Gene Therapy. Working group members have also expressed their willingness to cooperate with organizations planning meetings or studies on human gene therapy.

Dr. Walters said Mr. Rogers had also questioned whether the working group has sufficiently broad expertise to deal with all of the dimensions of human gene therapy. Dr. Walters said it is difficult for a group the size of the Working Group on Human Gene Therapy to cover all the possible bases, but the working group is committed to enlisting the help and comments of consultants to try and obtain the broadest spectrum of opinion.

Dr. Walters said Mr. Rogers had also suggested the working group, utilizing an interdisciplinary approach, should begin the difficult task of addressing "risk-benefit and value-resolution issues." Dr. Walters said the working group agreed with this suggestion. The points to consider document attempts to indicate specific concerns. The working group expects to learn a great deal from investigator responses to these specific points, and as experience is gained it will be possible not only to ask questions but to indicate the expectations of the working group and the RAC.

Dr. Walters asked RAC to approve these points to consider as a guidance document for researchers considering the application of gene therapy to patients. Dr. Gottesman emphasized that the points to consider document is a working document which will change as the technology develops. The points to consider are not intended for inclusion in the NIH Guidelines.

Dr. Davis said the working group did an extraordinarily good job of thoroughly analyzing the situation. He stressed that RAC's responsibility is to protect public safety; it is not to decide "what is good science." He did not wish to see RAC become "a fountain of orthodoxy for scientific research."

Dr. Johnson commended the working group for an excellent job. He expressed concern that some points may be too specific. He suggested the word "may" be substituted for the word "will" in Section I-D-5-a. Dr. W. French Anderson of the NIH, a member of the Working Group on Human Gene Therapy, said Dr. Johnson's suggested modification is a good one since at this stage it is difficult to predict which effects will be reversible. Dr. Walters accepted Dr. Johnson's suggested modification as the fifteenth technical change to the points to consider document.

Dr. Walters said RAC should address the issue of whether the NIH Guidelines and the points to consider document should explicitly cover recombinant RNA since the RNA-containing retroviruses will almost certainly be the vectors used in the first human gene therapy protocols. Several RAC members agreed.

Dr. Talbot said under the aegis of the NIH Guidelines, the points to consider document provides guidance on the type of information to be submitted for review of human gene therapy protocols. Procedurally, the points to consider document should not refer to recombinant RNA when the NIH Guidelines do not specifically refer to recombinant RNA. The NIH Guidelines can be modified after publication of the proposal in the Federal Register for comment, review, and recommendation by the RAC and approval by the NIH Director.

Dr. Walters suggested a generic proposal to modify the NIH Guidelines to refer to recombinant RNA be brought to RAC at the January 1986 meeting. He said the Working Group on Human Gene Therapy could then offer a proposal to modify footnote one of the points to consider document to explicitly address recombinant RNA.

Dr. Clowes suggested the first footnote is superfluous if no reference to recombinant RNA can be made; the footnote should be deleted. Drs. Talbot and Gottesman said the first footnote indicates DNA derived from recombinant DNA is also covered by the points to consider document; Section III-A-4 explicitly indicates the NIH Guidelines cover DNA derived from recombinant DNA. This section applies to human gene therapy protocols in which restriction enzymes would be used to excise the cloned "foreign DNA" from the recombinant vector.

Dr. Walters explained that in an attempt to perform gene therapy in 1980, the investigator proposed treating the recombinant DNA with restriction nuclease and administering the resulting DNA fragments to the patients. The working group wishes to make clear that this type of procedure is now covered by Section III-A-4 of the NIH Guidelines and by the points to consider document.

Dr. Davis suggested the language in the first footnote might be improved. Several RAC members agreed.

Dr. Wensink asked whether the working group might be inappropriately "second guessing" IRBs by dealing with issues such as selection of patients, informed consent, privacy, and confidentiality which generally fall within IRB purview.

Dr. Walters said the working group evaluated the general regulations on research involving human subjects and cited those sections which have particular applicability to human gene therapy. The working group does not request specific answers but simply asks IRBs and investigators to think about a series of questions. The working group is not trying to second guess the IRBs; it is only attempting to have the same general questions considered by all groups. Dr. Davis asked whether language should be inserted in the points to consider document stating that the working group is not attempting to supplant IRB functions.

Dr. Gottesman said Drs. Davis and Wensink had raised important questions concerning the relationship of the working group to IRBs. The working group has discussed this relationship at length. The working group does not wish to second guess IRBs but wishes to be certain every issue is properly considered particularly in the initial protocols.

Dr. Walters said it is difficult to predict how local IRBs will approach gene therapy issues. However, the Introduction of the points to consider document indicates flexibility by stating that not every point in the document will require attention in every proposal.

Dr. Johnson said RAC and the working group are not regulatory bodies and the points to consider are not regulations. These points are simply a listing of considerations relevant to human gene therapy.

Mr. Daloz said RAC and its working group will review proposals on a case-by-case basis. RAC and the working group will then evaluate how well the points to consider work in an actual review situation.

Dr. Landy asked why the section on "Applicability" was added to the document; he especially questioned the inclusion of the word "only." Dr. Gartland said this language was added at FDA's request to emphasize that NIH is not attempting to usurp FDA responsibilities.

Dr. Mary Ann Danello of the FDA said she was speaking for Dr. Henry Miller of the FDA. She asked that comments submitted by Dr. Miller on September 13, 1985 (tab 1243), be reconsidered especially his points four, five, six, and twelve.

Dr. Walters said these four points were originally offered in Dr. Miller's February 20, 1985, letter commenting on the January 22, 1985, Federal Register announcement. Those comments were fully discussed at the April 1, 1985, meeting of the working group. Comment six was accepted by the working group at that time and the points to consider document modified. On April 1, 1985, a majority of the working group decided not to accept suggestions four, five, and twelve. Dr. Miller again offered these comments in response to the August 19, 1985, Federal Register. The subworking group again considered these comments on September 20, 1985, and rejected them. Dr. Walters said the working group fairly considered all the issues raised in the comment letters.

Mr. Jeremy Rifkin of the Foundation on Economic Trends thanked Dr. Walters and the working group for the consideration shown to the concerns expressed by the Foundation on Economic Trends.

Mr. Rifkin said the points to consider document demonstrates careful consideration and thought and contains a great deal of technical detail; however, "short shrift" was given to social issues. He thought the points to consider lack guidance for evaluating ethical and social issues. He suggested society should begin to wrestle with social and ethical questions with the same degree of careful consideration given to technical issues. He said "not to do so would be to show a lack of responsibility for the tremendous weight of this technology."

Mr. Rifkin said "it is not too early to ask some long-term questions and to develop some protocols about the eugenic implications" of gene therapy. He contended that failure to address ethical and social issues associated with germ line modification and the relationship between somatic-cell and germ line therapy will delay the review process. He drew an analogy to field testing of modified organisms. Mr. Rifkin said appropriate review protocols had not been prepared by the RAC Working Group on Release into the Environment and RAC was "ill-equipped" during the first reviews to "ask the appropriate questions. As a result, there's been in a two year delay in deliberate release."

Mr. Rifkin said criteria should be established for determining what types of somatic-cell therapy would not be permissible. He thought language in the Federal Register announcement implies "that enhancive gene therapy would only come up in germ line experiments which is not really true."

He thought it possible the working group will receive a proposal dealing with "enhancive somatic-cell gene therapy, not just gene therapy that relates to a disease."

Mr. Rifkin contended that human gene therapy is "not just an extension of normal medical technology. In many ways, it has similarities; but also as you know, it's qualitatively different because we're now looking at changing genetic instructions in the code for human beings."

Dr. Walters said different issues arise in considering unintended side effects of somatic-cell human gene therapy than are raised by deliberate germ line interventions. The working group thinks it unlikely inadvertent germ line effects will occur in somatic cell gene therapy since somatic-cell gene therapy is analogous to organ transplantation. However, the Working Group on Human Gene Therapy is asking investigators to look very carefully for unintended germ line effects in pre-clinical studies. Dr. Walters said even if unintended effects on the patients' germ line occurred, such effects would simply be one factor in the overall risk/benefit calculation for a therapy which would most likely be administered to children who would otherwise die in the near future.

Dr. Walters said the first proposals would involve treatment of disease and not enhancive gene therapy. The points to consider document specifically states in Section I-A-1 "why is the disease selected for treatment...."

Mr. Rifkin suggested the composition of the working group be reexamined and the expertise of this working group broadened to include theologians, anthropologists, and psychologists and a "wide range of people." He also said the membership of the RAC should be expanded. He argued that human gene therapy is a social and cultural issue as well as a medical issue, and the precedents set in the next five years will influence developments in this area for the next 25 years. He said "it's not good enough just to have consultants...."

Dr. Walters said the Working Group on Human Gene Therapy is caught between opposing camps of critics on this issue; some critics state the working group needs more technical members while other critics state the working group needs greater representation in ethics, laws, and the social sciences. The working group has tried to strike a balance; it consists of approximately four laboratory scientists, three clinicians, three ethicists, three lawyers, and two public policy experts. The current members of the working group are individuals who have published for the past fifteen years on the technical, ethical, legal, and social issues surrounding human gene therapy. Dr. Walters said as experience is accumulated, the working group may discover it requires expertise in other areas and could be augmented with additional members should the need become apparent. However, how

large a committee can become and still function effectively is an important consideration.

Dr. Davis expressed concern about the analogy which Mr. Rifkin drew between the activities of the Working Group on Release into the Environment and the Working Group on Human Gene Therapy. He felt ecologists can offer pertinent technical advice and should participate in reviews of experiments involving release of modified organisms into the environment. He questioned, however, whether theologians are in a position to give equally pertinent society-wide answers in the matter of morals.

Mr. Rifkin said theologians would "have a lot to say that would be very important about the question of where the human race goes from here." Scientists can perform a useful role in educating theologians, and theologians can inform scientists about the larger ethical and social questions.

Dr. Walters said two members of the Working Group on Human Gene Therapy have theological training; one is a member of the Department of Religious Studies at the University of Virginia.

Dr. Landy said no one group appears to have unique insights to contribute to the discussion of human gene therapy. The issues in human gene therapy are matters for broad public discussion.

Mr. Mitchell said the points to consider have been widely distributed for public comment. The document was published twice in the Federal Register, and a special effort was made to distribute it to individuals who expressed interest in these issues.

Dr. Walters moved that RAC adopt the points to consider as published for comment in the August 19, 1985, Federal Register, with the fourteen technical amendments proposed by the Subworking Group on Human Gene Therapy plus the two technical amendments offered by RAC (Attachment II). Dr. Gottesman seconded the motion.

By a vote of seventeen in favor, none opposed, and no abstentions, the RAC adopted the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols."

IV. BIOTECHNOLOGY SCIENCE BOARD

Mr. Mitchell introduced Dr. Bernadine Healy, the Deputy Director of the Office of Science and Technology Policy (OSTP) of the Executive Office of the President. Mr. Mitchell said Dr. Healy is the Chair of the Cabinet Council Working Group on Biotechnology that issued the December 31, 1984, Federal Register proposal (tab 1242) for a coordinated framework for biotechnology regulation and oversight. Mr. Mitchell said the Cabinet Council

Working Group on Biotechnology has recently been restructured into a working group of the Domestic Policy Council.

Dr. Healy began by stating that:

> "The importance of the RAC to this area, both nationally and internationally, is undisputed; and it is everyone's expectation and hope that the NIH RAC will continue to exist and play a vigorous role."

Dr. Healy said a number of comments were received in response to the December 31, 1984, Federal Register proposal. The primary concern expressed in these comments was that the proposed two-tier scientific review mechanism would be too cumbersome, slow, and rigid. Industry was also concerned about confidentiality. The Domestic Policy Council Working Group on Biotechnology thus attempted to develop an alternative framework.

Dr. Healy said several important issues had to be considered in developing a scientific review mechanism: (1) Redundant reviews should be avoided. (2) A coordinated scientific advisory system should be aware of the issues faced by the various agencies. (3) Confidentiality and statutory requirements render it difficult for a group with extramural membership to review all applications. (4) A central review board probably would not be able to meet frequently enough to review in detail every application submitted to every agency.

Dr. Healy said one proposed structure involved a "super-RAC," a single scientific advisory committee similar to the NIH RAC, to be located in the Office of the Assistant Secretary for Health (ASH) of the Department of Health and Human Services (DHHS). The "super-RAC" would coordinate biotechnology issues across all Federal agencies. Some commentors suggested the NIH RAC be transformed into the "super-RAC."

Dr. Healy said the "super-RAC" proposal was widely discussed and received a certain amount of support within the Domestic Policy Council Working Group. However, a "super-RAC" structure was not feasible for several reasons: (1) The RAC meets only about four times a year and would not be able to respond to the statutory time requirements of several of the agencies. (2) Confidentiality problems would be almost insurmountable for a committee such as RAC. (3) RAC may not possess the expertise needed to deal with some of the proposals submitted to regulatory agencies. RAC would have to be substantially modified or at the very least would have to be augmented in order to be transformed into a "super-RAC."

Dr. Healy said a variation of the super-RAC proposal would have created a RAC clone in ASH; the RAC would have remained at the NIH. The obvious question, however, that arises from such an arrangement is "who's on first."

How do these similar, but not equal groups deal with issues? How do they divide up their responsibilities? Which committee would be the deliberating body? Preempted by its clone the time-honored, established, widely-respected, working NIH RAC could wither on the vine.

Dr. Healy said the Domestic Policy Council Working Group on Biotechnology felt these concerns were legitimate. Another oversight plan was then developed and unanimously accepted by the working group.

Dr. Healy said this plan is similar in intention to the plan described in the December 31, 1984, Federal Register, and to quote from that document the objectives are:

> "...to provide expert advice on scientific issues related to the approval of biotechnology products and research applications; to provide a coordinating forum for addressing scientific problems, sharing information and for consensus building; to promote consistency in the development of agencies' review procedures and assessments; to promote continuing cooperation among Federal agencies on emerging scientific issues; and, to identify gaps in knowledge."

Dr. Healy said the coordinating focus of this proposed oversight system will be under a statutory interagency committee, the Federal Coordinating Council for Science, Engineering, and Technology (FCCSET). Dr. Healy said OSTP has established several FCCSET committees for issues requiring interagency coordination. The FCCSET committee for research and commercial applications of biotechnology will be the Biotechnology Science Coordinating Committee (BSCC). FCCSET committees are administratively housed within OSTP and officially chaired by the OSTP Director although the working Chair is usually another Federal official. In the case of the BSCC, it has been suggested that the Chair might appropriately alternate between the directors of the National Science Foundation (NSF) and the NIH.

Dr. Healy said the BSCC will coordinate activities; it will not regulate. Regulatory authority resides in the regulatory agencies.

Dr. Healy said each agency will have its own scientific review mechanism since each of the agencies have their own statutory obligations and responsibilities. The NIH will have its RAC; the U.S. Department of Agriculture will continue with a committee like its Agricultural Recombinant DNA Research Committee; the Environmental Protection Agency (EPA) has formed a subcommittee of its scientific advisory panel; and the FDA has its own established scientific review procedures. Each agency will be responsible for identifying and obtaining the appropriate scientific expertise.

Dr. Healy said the BSCC will not review proposals but will coordinate and develop a consensus on interagency policy and science issues. The BSCC

should be aware of agency activities and promote consistency and consensus in scientific considerations. The BSCC will evaluate review procedures developed by agency-based scientific advisory committees, analyze broad scientific issues, and develop generic scientific guidelines. Confidentiality issues will be simplified since BSCC members are government employees who will review redacted material; confidential information can be protected under the confidentiality procedures of each member agency.

Dr. Healy said FCCSET committees exist when there is a need; she imagined the BSCC will no longer be necessary once the review system is functioning.

Dr. Healy said under this plan the NIH RAC will continue to perform the function it has filled in the past. It might in addition serve as a resource for other government agencies. The BSCC will not preempt RAC.

Dr. Healy stressed that NIH RAC is the model of an effective scientific advisory mechanism. It has functioned well over the past ten years, and constitutes an important process which should be preserved and continued. RAC is expected to continue to play a vigorous role in future oversight structures.

Dr. Mitchell asked whether the BSCC will distinguish between research and commercial applications. Dr. Healy said research applications would be expected to be submitted primarily to NIH and NSF while commericial applications would go to the regulatory agencies. The BSCC may help route applications appropriately.

Dr. Martin expressed concern that this type of regulation of biotechnology could "hinder technology development and the application of biotechnology to societal needs and economic benefits." He thought this new technology would be more effectively encouraged through an educational process rather than an oversight mechanism. Dr. Healy said this proposed scientific advisory mechanism could be involved in educating the public.

Dr. Davis said the BSCC sounds reasonable but may have little to do since the agency-based review committees may be able to handle all the problems. Dr. Pramer said some biotechnology proposals may fall under the jurisdiction of more than one agency. Does a mechanism for mediation and arbitration exist under the FCCSET mechanism? Would a conflict be mediated by the BSCC? Dr. Cohen asked how disagreements would be resolved.

Dr. Healy said disagreements occur in the government when uncertainties exist over agency jurisdiction and are usually worked out by the agencies in an informal good-faith manner on a case-by-case basis. The BSCC will not become involved in jurisdictional "turf battles" between agencies but

will coordinate consistent scientific advice across the agencies. The BSCC is not an oversight council with statutory authority; it can not tell an agency how to proceed. The BSCC has no authority to tell an agency to change its views, its review structure, or its regulatory decisions. Under the FCCSET mechanism, it is conceivable all the agencies could adopt the same scientific guidelines but arrive at very different regulatory decisions because of the statutes under which they operate. The BSCC will identify problems and can provide a forum for the agencies to resolve differences.

Dr. Walters questioned the ability of a FCCSET committee to communicate with the public and the agency committees. Will BSCC agendas be public; will minutes of the meetings be public?

Dr. Healy said FCCSET committee meetings are not generally open to the public; however, the agencies will develop policies through agency approval processes which usually provide for public comment and participation. Meetings of the various agency scientific advisory committees may be open to the public depending on the needs of the agency. The FDA or EPA committees are more likely to meet in closed sessions to deal with confidential matters. The NIH and NSF committees will generally function in open session.

Dr. Walters asked whether committees such as RAC would be aware an issue is being considered by the BSCC and if a mechanism exists whereby RAC would have an opportunity to provide input on an issue. Dr. Healy said it will be the responsibility of the agency to keep its advisory group and the public informed of BSCC activities and actions.

Dr. Rapp expressed concern that five independent RAC-like groups operating under different auspices might arrive at different conclusions on the same issue and send mixed signals to the public. In such a situation, the public will be confused and its confidence undermined. Dr. Rapp said he did not see how the FCCSET mechanism could avoid this confusion. On theoretical grounds he thought it best to have one broad-based review committee.

Dr. Healy said it is expected that EPA and FDA will be dealing primarily with individual commercial applications that will be confidential. RAC will continue to deliberate on generic issues and will continue to be preeminent in public discussions.

Dr. Johnson was also concerned about public confidence. The BSCC will deal with generic scientific issues but will not be open to the public. The degree of public participation provided by RAC will not be provided by the BSCC. Recent samplings of public confidence show the public is not greatly concerned over biotechnology. He attributed this attitude to the existence of RAC as a forum for public participation and scientific input.

Dr. Healy said many opportunities for public input will exist under the FCCSET system. The FDA and EPA advisory panels hold open sessions. The NIH and the NSF advisory panels are generally open to the public. The public will have input into policy at the agency level.

Dr. Johnson said in his perception public input was being diluted rather than expanded by the BSCC proposal. Dr. Healy asked whether Dr. Johnson could suggest an alternative mechanism. Dr. Johnson said RAC has provided a system of scientific oversight which has worked reasonably well; no additional regulation of the products of the technology beyond what already exists is required.

Dr. Healy said the existing RAC review system cannot address the need for scientific advice on commercial applications in the short time frame specified by certain regulatory statutes. In addition, some mechanism to coordinate the scientific advice offered to the various agencies is necessary. The BSCC is an attempt to provide a mechanism for communication among agencies. Dr. Healy said industry wants a streamlined, coordinated, consistent review system; however, it appears to resist this FCCSET attempt to promote consistency on scientific issues across agencies.

Dr. W. French Anderson asked how the national ethics commission proposed by Senator Albert Gore (D-Tenn.) would interact with the BSCC. Dr. Healy said the BSCC is a coordinating committee for scientific issues; it is not anticipated the BSCC will address ethical issues.

Mr. Mitchell asked whether an agreement has been signed by the agencies stating their intention to work cooperatively under the FCCSET mechanism. Dr. Healy replied that such an agreement would soon be signed.

Mr. Mitchell thanked Dr. Healy for speaking with RAC on this issue.

V. PROPOSED AMENDMENTS OF APPENDIX C-III

Mr. Mitchell called upon Drs. David Glass and R. Rogers Yocum of BioTechnica International, Inc., to describe their proposal (tabs 1235/III, 1239) to amend Appendix C-III of the NIH Guidelines.

Under this proposal the first paragraph of Appendix C-III would be amended to read as follows:

> "Experiments which use Saccharomyces cerevisiae host-vector systems, with the exception of experiments listed below, are exempt from these NIH Guidelines."

This amendment would extend the current exemption to strains other than "laboratory strains" of Saccharomyces cerevisiae.

A second new paragraph would be added to Appendix C-III to read as follows:

> "Experiments which use Saccharomyces uvarum host-vector systems, with the exception of experiments listed below, are exempt from these NIH Guidelines."

Drs. Glass and Yocum argued there is no clear distinction between laboratory and industrially useful strains of Saccharomyces cerevisiae by biochemical and morphological criteria. Laboratory strains are derived from wild and industrial strains and are therefore genetically closely related to these strains. The arguments supporting the exemption of laboratory S. cerevisiae strains from the NIH Guidelines (e.g., nonpathogenicity) apply to industrial strains and warrant the extension of exempt status to S. uvarum.

Drs. Glass and Yocum proposed that all strains classified as Saccharomyces uvarum (including those designated S. carlsbergensis which have recently been reclassified as Saccharomyces uvarum) be given exempt status.

Dr. Yocum said S. uvarum is used in the production of lager beer while S. cerevisiae is used for producing ale and wine, in distilling and in baking, although some distillery strains are classified as S. uvarum.

Dr. Yocum distributed data which demonstrated greater variability among the S. cerevisiae strains than between the S. uvarum strains and the S. cerevisiae strains.

Dr. Mortimer said S. cerevisiae and S. uvarum are very similar.
Dr. MacQuillan said it appears that S. uvarum strains are very closely related to S. cerevisiae strains and that laboratory strains of S. cerevisiae are very similar to industrial strains. However, the molecular and genetic evidence supporting this observation is limited since only portions of the genomes of these organisms have been analyzed.

Dr. Davis said no Saccharomyces has ever been shown to be pathogenic for man or animals or to colonize man. He could not imagine the environment or human beings being threatened by any recombinant Saccharomyces unless toxin genes were introduced. Dr. Davis suggested the NIH Guidelines be revised to exempt all Saccharomyces species. Dr. Cohen agreed.

Dr. Gottesman said historically RAC has evaluated several issues in determining appropriate containment. Pathogenicity is but one of the issues. Another area of concern has been dispersal of recombinant DNA through novel routes of transfer.

Dr. MacQuillan agreed an organism certifiable as S. cerevisiae should be exempt under Appendix C of the NIH Guidelines. He was not certain, however, that all Saccharomyces strains should be exempted as proposed by Dr. Davis. Dr. MacQuillan said the issue is the difficulty in identifying and defining Saccharomyces strains.

Dr. Mortimer explained it is almost impossible to do genetics on most industrial strains. Some strains are triploid or higher ploidy. Industrial strains usually either fail to sporulate or, if they do sporulate, the spores have extremely low viability. Physiological and morphological criteria must therefore be used for identification.

Dr. Talbot doubted the NIH Director would approve a recommendation to exempt all Saccharomyces species since such a proposal had not been published in the Federal Register for public comment.

Dr. Pramer moved acceptance of the BioTechnica International, Inc., request as it appeared in the August 19, 1985, Federal Register. The motion was seconded by Dr. Cohen.

By a vote of seventeen in favor, none opposed, and no abstentions, the RAC recommended acceptance of the proposal as it appeared in the Federal Register.

Dr. Walters suggested RAC might establish a working group to evaluate the proposal to exempt all Saccharomyces species from the NIH Guidelines. Drs. Davis and Pramer did not favor establishment of such a working group at this time.

VI. PROPOSED AMENDMENT OF PART III OF THE NIH GUIDELINES

Dr. Johnson said Dr. Bernard Talbot, Deputy Director of the National Institute of Allergy and Infectious Diseases, in a memorandum (tab 1240) dated February 12, 1985, noted that under the NIH Guidelines certain proposals are received for review by the NIH RAC and approval by NIH. Recently other Federal agencies have taken steps toward assuming new roles in the review of recombinant DNA proposals. Because of these developments, a proposal submitted to the NIH for RAC review and NIH approval may also be submitted to another Federal agency for review.

Dr. Talbot had written:

> "In such a case, I believe it would be advantageous for NIH to have the option of deferring to the review and approval by the other Federal agency rather than always going through review and approval by both the other Federal agency and the NIH. In order to allow this, I request that the following proposed change in the NIH Guidelines be issued for comment, and placed on the agenda of the next RAC meeting. I propose that a new sentence be added at the end of Section III-A of the Guidelines ('Experiments that Require RAC Review and NIH and IBC Approval Before Initiation') just before Section III-A-1 of the Guidelines as follows:

'If experiments in this category are submitted for review to another Federal agency, the submitter shall notify ORDA; ORDA may then determine that such review serves the same purpose, and based on that determination, notify the submitter that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal Agency.'"

Dr. Johnson said this proposal had originally been discussed by RAC at the May 3, 1985, meeting, and tabled. Dr. Talbot said Dr. Miller of the FDA had informed RAC at the May 3, 1985, meeting that he would submit for the September 23, 1985, RAC meeting a proposal requiring, rather than only permitting, NIH to defer to other agencies in such cases. Dr. Miller has not submitted such a proposal. Dr. Landy said the only reason RAC tabled Dr. Talbot's proposal at the last meeting was because it anticipated receiving an FDA proposal and preferred to evaluate both proposals at the same time.

Dr. Pramer said it was very important to act on Dr. Talbot's proposal in light of the earlier presentation by Dr. Healy.

Dr. Davis asked if a codicil could be added to Dr. Talbot's proposal suggesting that other agencies could defer to RAC. Dr. Talbot said it would not be appropriate to put such a statement in the NIH Guidelines, but it could be suggested informally to other agencies.

Dr. Clarence Styron of Monsanto Company asked whether Dr. Talbot's proposal would affect IBCs. Dr. Talbot said Institutional Biosafety Committees would retain their functions; they would not defer to other agencies.

Dr. Walters moved RAC accept the proposal offered by Dr. Talbot. Dr. Rapp seconded the motion.

By a vote of seventeen in favor, none opposed, and no abstentions, the RAC accepted Dr. Talbot's proposal.

VII. REQUEST TO CLONE SHIGA-LIKE TOXIN FROM ENTEROBACTERIACEAE AND VIBRIONACEAE

Dr. Gottesman recounted the history of the proposal (tabs 1229, 1235/I, 1236, 1238) to clone the Shiga-like toxin from Enterobacteriaceae and Vibrionaceae. Dr. Alison O'Brien of the Uniformed Services University of the Health Sciences had previously (49 FR 36052) received permission from NIH to clone the structural gene of the Shiga-like toxin (SLT) of Escherichia coli in E. coli K-12. The conditions under which the gene may be cloned are described in Appendix F-IV-H of the NIH Guidelines as follows:

"The intact structural gene(s) of the Shiga-like toxin from E. coli may be cloned in E. coli K-12 under BL3 + EK1 containment conditions.

"E. coli host vector systems expressing the Shiga-like toxin gene may be moved from BL3 to BL2 containment conditions provided that: (1) the amount of toxin produced by the modified host-vector systems be no greater than that produced by the positive control strain 933 E. coli 0157:H7, grown and measured under optimal conditions; and (2) the cloning vehicle is to be an EK1 vector preferably belonging to the class of poorly mobilizable plasmids such as pBR322, pBR328 and pBR325.

"Nontoxinogenic fragments of the Shiga-like toxin structural gene(s) may be moved from BL3 + EK1 to BL2 + EK1 containment conditions or such nontoxic fragments may be directly cloned in E. coli K-12 under BL2 + EK1 conditions provided that the E. coli host-vector systems containing the fragments do not contain overlapping fragments which together would encompass the Shiga-like toxin structural gene(s)."

In a letter dated July 26, 1985, Dr. O'Brien subsequently requested permission to clone SLT structural genes, defined either by nucleotide sequence homology with SLT gene probes from E. coli or by antigenic cross-reactivity of their gene products with purified E. coli SLT, from bacterial species classified in the familes Enterobacteriaceae or Vibrionaceae into E. coli K-12 under conditions similar to those specified in the previous permission. Dr. O'Brien requested permission to broaden the scope of study of SLT toxin for the following reasons: (1) to investigate the potential role of SLT in the pathogenicity of a variety of bacteria; (2) to compare the SLT genes from various pathogenic bacteria; and (3) to facilitate the development of an experimental Vibrio cholerae vaccine deleted for SLT genes.

Dr. Gottesman said the RAC Working Group on Toxins reviewed the July 26, 1985, proposal on August 16, 1985, and offered three recommendations to RAC: (1) The working group first recommended by a vote of six in favor, none opposed, and no abstentions that the proposal as it appeared in the Federal Register of August 19, 1985 be approved. (2) The working group then recommended by a vote of six in favor, none opposed, and no abstentions that Campylobacter strains be considered as members of the Vibrionaceae for purposes of this proposal. (3) By a vote of five in favor, one opposed and no abstentions, the working group recommended that Shigella dysenteriae 60R strain be substituted in Appendix F-IV-H for 933 E. coli 0157:H7; as the reference strain against which toxin production is measured to determine whether the clone can be removed from BL3 containment.

Drs. Collier and Clowes concurred with the recommendations of the Working Group on Toxins.

Dr. Collier pointed out that investigators routinely work under BL2 containment conditions with pathogenic Shigella dysenteriae strains producing the maximum amount of toxin seen in strains in nature. He said it is not reasonable to restrict E. coli K-12 host-vector systems expressing the

Shiga-like toxin to containment higher than used with naturally occurring Shigella dysenteriae; the E. coli K-12 host-vector systems do not have the attributes of pathogens.

Dr. Cohen said there is no reason to expect E. coli host-vector systems expressing the Shiga-like toxin gene would have any greater pathogenic potential than Shigella dysenteriae strains; it would be reasonable to handle the E. coli host-vector systems under the same conditions used to handle Shigella dysenteriae.

Dr. Davis noted that nomenclature is not as sharp among lower prokaryotic organisms as it is among higher organisms. Campylobacter in previous editions of Bergey's Manual of Systematic Bacteriology were classified as Vibrionaceae.

Dr. Gottesman moved that RAC accept Dr. O'Brien's proposal as published in the August 19, 1985, Federal Register with Campylobacter species considered to be Vibrionaceae. Dr. Cohen seconded the motion.

By a vote of seventeen in favor, none opposed, and no abstentions, the RAC adopted the motion.

Dr. Gottesman explained the working group motion to substitute Shigella dysenteriae 60R for E. coli 0157:H7 in Appendix F-IV-H. Appendix F-IV-H specifies that E. coli K-12 host-vector systems expressing Shiga-like toxin may be moved from BL3 to BL2 containment if these systems express no more toxin than standard strain 933 E. coli 0157:H7 grown and measured under optimal conditions. Substitution of Shigella dysenteriae 60R for 933 E. coli 0157:H7 raises by a factor of ten the level of toxin the host-vector system may produce and still be removed from BL3 containment. E. coli standard strain 0157:H7 under optimal conditions produces approximately 10^7 cytotoxic doses (CDs) per mg protein in cell lysate; Shigella dysenteriae strain 60R produces 10^8 CDs per mg protein. The issue was whether it would be appropriate to specify as a standard a strain expressing the maximum amount of toxin expressed under optimal conditions by Shigellae found in nature.

The primary issue for working group members was how safe is safe enough. Some members of the working group preferred to consider toxin toxicity as the primary criterion while other members of the group wished to also consider the characteristics of the host-vector system.

Dr. Davis asked whether the standard should be the behavior of an organism, which would vary, rather than an absolute number. Dr. Gottesman said designating a bacterial strain as an internal standard is appropriate. An internal standard guarantees that individuals who handle the clones well

and obtain maximum production would not be penalized compared to individuals who do not obtain maximal production.

Dr. Gottesman moved that Shigella dysenteriae 60R be substituted for 933 E. coli 0157:H7 as the standard strain in Appendix F-IV-H. Dr. Clowes seconded the motion.

By a vote of seventeen in favor, none opposed, and no abstentions, the motion was accepted by RAC.

VIII. REQUEST TO CLONE A HYBRID TOXIN GENE

Dr. Gottesman said Dr. John Murphy of the University Hospital of Boston University Medical Center requested permission (tabs 1230, 1233, 1235/II, 1236, 1237) to construct a hybrid molecule in which the gene coding for interleukin-2 (IL-2) is joined to a segment of the gene encoding diphtheria toxin. The diphtheria toxin segment encodes the A subunit and portions of the B subunit of the toxin molecule. The hybrid gene would be cloned in E. coli K-12 host-vector systems.

Dr. Gottesman said the Working Group on Toxins at the August 16, 1985, meeting had considered this proposal as well as a second related proposal dealing with a hybrid toxin composed of α-melanocyte stimulating hormone (α-MSH) and a diphtheria toxin segment.

Dr. Gottesman reported the history of the α-MSH-diphtheria toxin proposal. The α-MSH-diphtheria toxin proposal had been reviewed and recommended for approval by RAC at the October 25, 1982, meeting, and Dr. Murphy had been granted NIH permission (Appendix F-IV-I, 49 FR 46279) to propagate under BL4 containment the hybrid gene coding for the α-MSH-diphtheria toxin fusion protein. The permission required Dr. Murphy to submit data to the Working Group on Toxins for evaluation in order to obtain permission to remove the clones from BL4 containment. Dr. Murphy accordingly submitted data from risk assessment experiments to the Working Group on Toxins for review.

These data described the effects on guinea pigs challenged by: (1) intraperitoneal injection of the viable and boiled E. coli K-12 host-vector system (the control); (2) intraperitoneal injection of the viable and boiled E. coli K-12 host-vector system carrying and expressing the hybrid gene; and (3) injection of partially purified fusion protein at graded doses up to 1000 minimal lethal dose equivalents (based on 160 nanograms diphtheria toxin per kilogram body weight as minimal lethal dose.) Dr. Murphy argued these data suggest E. coli host-vector systems carrying the hybrid gene are not harmful to guinea pigs, and that up to 1000 equivalent diphtheria toxin doses of fusion protein challenges to guinea pigs cause no effect. After thorough discussion, the working group on August 16, 1985, recommended Dr. Murphy be permitted to proceed with experiments involving clones

carrying the α-MSH-diphtheria toxin gene at BL2 containment with BL3 practices. That recommendation was sent to the NIH Director for his approval.

Dr. Gottesman said the concerns hypothesized by the working group for the IL-2-diphtheria toxin proposal and the α-MSH-diphtheria toxin proposal were similar; i.e., what are the target cells; what are the implications of a host-vector system producing such hybrid toxins; what is the toxicity of the diphtheria toxin fragment?

Dr. Gottesman said the goal of the IL-2 proposal is the development and testing of novel therapeutic agents to combat organ rejection and/or create a state of tolerance in organ transplantation. As a by-product, some of the agents may have therapeutic potential for treatment of leukemia and lymphoma.

Dr. Waldmann said antigens binding to T cell surface receptors activate T cell function. Activation initiates second-stage events, including the production of IL-2. IL-2 interacts with high affinity receptors on the surface of certain cells. Resting T cells generally do not possess IL-2 receptors. Dr. Waldmann said the only cells expressing IL-2 receptor are the activated T lymphocytes in lymph nodes and tonsils.

Dr. Waldmann said in some circumstances physicians might wish to eliminate IL-2 expressing cells; e.g., in adult T-cell leukemia, in transplantation of mismatched organs, or in certain autoimmune states such as aplastic anemia. Dr. Waldmann said it would be very useful to have IL-2-diphtheria toxin conjugates for clinical use. These conjugate proteins would probably not be more toxic than diphtheria toxin itself.

Dr. Waldmann said patients with aplastic anemia and with adult T cell leukemia have been treated with antibody against the IL-2 receptor. While the numbers of IL-2 expressing malignant cells have been reduced, no complications such as reduction in numbers of platelets, granulocytes, or normal T cells have been noted. Dr. Waldmann said a patient has also been treated with a hybrid protein composed of Pseudomonas exotoxin and antibody to the IL-2 receptor. In this case, a slight reduction in number of granulocytes was observed but no other toxicity. He hypothesized that mature T cells, marrow and thymus cells as well as their precursors, would not be damaged by a ten day exposure to the IL-2-diphtheria toxin fusion protein.

Dr. Clowes noted that the Working Group on Toxins recommended use of EK2 vectors; he questioned why the working group had not recommended use of EK2 hosts as well for these procedures. He felt use of these hosts might offer some additional measure of security.

Dr. Gottesman said it is more difficult to work with the debilitated EK2 hosts. The working group had not actually recommended the use of EK2 certified vectors but had recommended the use of poorly mobilizable vectors such as the EK2 vectors.

Dr. Clowes asked Dr. Murphy why he used a morphological test for toxicity testing rather than a more specific quantitative protein inhibition assay.

Dr. Murphy said he has recently employed protein inhibition tests to show that protein synthesis in human malignant melanoma cells is inhibited 50 percent by approximately 2 nanomoles of the α-MSH-diphtheria toxin fusion protein. Diphtheria toxin sensitive Chinese Hamster Ovary cells are not affected by the hybrid toxin protein. Dr. Murphy said the fusion protein appears to be directed only against cells having α-MSH receptors on their surface.

Dr. Cohen asked how the fact most of the U.S. population is immunized against diphtheria would affect the therapeutic effectiveness of the hybrid protein.

Dr. Murphy said two years ago his group published a monoclonal antibody study of the interaction of 43 different monoclonal antibodies with diphtheria toxin. The study demonstrated that only those monoclonal antibodies that prevented diphtheria toxin from binding to its receptor were neutralizing antibodies. Antibodies binding to other portions of the toxin molecule did not neutralize cytotoxic activity. Preliminary experiments with polyclonal antisera show that concentrations more than 100-fold greater than would be expected to neutralize diphtheria toxin activity are needed to reduce the potency of the α-MSH-diphtheria toxin fusion protein on human malignant melanoma cells.

Dr. Davis asked whether the diphtheria toxin fragment used to construct the fusion protein has any toxicity.

Dr. Murphy said the fragment codes for all of diphtheria toxin except for 67 amino acids which have been deleted from the carboxyl-terminal end. It is not currently known whether the diphtheria toxin receptor region.is in that 67 amino acid section or whether that region is necessary to maintain the appropriate configuration for receptor binding. However, the fusion protein in which the carboxyl-terminal 67 amino acids are replaced with a 13 amino acid sequence of α-MSH is not cytotoxic for cells exquisitely sensitive to diphtheria toxin. He inferred from this observation that the carboxyl-terminal region carries either the binding domain or segments necessary for the appropriate molecular configuration.

Dr. Martin asked Dr. Murphy whether he had tested the diphtheria toxin fragment without the α-MSH ligand for receptor activity and toxicity. Dr. Murphy said he had only studied the fusion protein. Dr. Martin said the ligand might affect toxin binding; the toxin fragment might be toxic if the ligand is removed.

Dr. Martin asked whether the carboxyl-terminal of the diphtheria toxin fragment had been modified other than by addition of the α-MSH ligand. Dr. Murphy said the carboxyl terminal end was modified; linker nucleotides were ligated to the diphtheria toxin fragment before the α-MSH ligand was added.

Dr. Martin suggested RAC might permit Dr. Murphy to test the toxicity of the diphtheria toxin fragment under BL2 containment; containment might then be lowered to BL2 for the fusion protein if the diphtheria toxin fragment is not toxic. Dr. Landy felt some questions would still remain unanswered; e.g., the fusion protein could be more easily processed than the toxin fragment alone. He felt enough was known now to permit the experiment to proceed.

Dr. Collier did not think containment higher than BL3 would be appropriate for procedures involving this hybrid toxin gene. He pointed out that the gene for Pseudomonas aeruginosa exotoxin A, a toxin only ten to fifty times less toxic than diphtheria toxin but with the same mode of action, may be cloned under BL1 containment conditions.

Dr. Collier noted that the Working Group on Toxins had suggested the use of BL2 containment with BL3 practices for studies involving the α-MSH-diphtheria toxin fusion gene. Dr. Rapp said he preferred to designate either BL2 or BL3 containment rather than BL2 containment with BL3 practices.

Dr. Gottesman said she would offer a motion but would abstain during the vote because she had discovered that investigators within her laboratory are working with IL-2. She moved that the request described in the August 19, 1985, Federal Register be permitted under BL3 conditions using E. coli K-12 hosts and poorly mobilizable plasmid vectors such as EK2 certified plasmids. There was no second to the motion.

Dr. Davis moved to substitute BL2 containment conditions plus BL3 practices in the motion offered by Dr. Gottesman. Dr. Clowes seconded the motion.

Dr. Walters felt the RAC appeared to be following a more liberal approach than the working group. He asked how a more restricted approach would affect the progress of the research.

Dr. Murphy said at present there is no functional BL3 laboratory in the city of Boston. If RAC recommends BL3 containment, a BL3 laboratory would have to be built.

Dr. Davis said RAC is responsible for promoting the public welfare by protecting the interests of patients as well as protecting society against hazard. He said:

"If I had a relative with malignant melanoma, I would be very unhappy if the availability of this material were delayed by half a year because a material that is not toxic to cells and is not toxic to animals still scares us because it has the word recombinant diphtheria toxin."

By a vote of twelve in favor, none opposed, and four abstentions, the RAC recommended the motion be accepted. Dr. Gottesman abstained.

IX. PROPOSED EXCHANGER LIST FOR GRAM POSITIVE BACTERIA

Dr. Gartland said this item (tab 1231) had not been published in the Federal Register for public comment. He said a RAC working group has been assembled to review the sublists of Appendix A pertaining to the gram positive Eubacteriales. A meeting of that working group has been scheduled for December 2, 1985.

Dr. Morris Levin of the EPA said the EPA Office of Toxic Substances had recently held a meeting dealing with microorganisms which exchange genetic information. A report on this meeting will soon be available.

X. FUTURE MEETING DATES

Dr. Gartland said the next RAC meeting is scheduled for January 27, 1986.

Dr. Walters asked that RAC meetings be scheduled three meeting dates in advance so the Working Group on Human Gene Therapy could key its meetings to appropriate times prior to RAC meetings. Investigators planning to submit gene therapy protocols could then be given deadlines for submitting materials.

Dr. Gartland said on occasions in the past, RAC meetings were scheduled a year in advance, and it is possible to arrange meetings in that fashion.

Dr. Walters suggested some portion of the next RAC meeting might be devoted to a "self-education process on gene therapy." Issues that might be addressed include: the status of retroviral vectors; the best candidate diseases for gene therapy; and what is known at present about the effects of somatic-cell gene therapy on the germ line.

XI. OTHER MATTERS

Drs. Gottesman and Tolin enquired about the status of the Working Group on Release into the Environment. Dr. Talbot said that no proposals for release into the environment have recently been submitted to NIH.

Dr. Pramer said the proceedings of the American Society for Microbiology symposium, "Engineered Organisms in the Environment: Scientific Issues," held in Philadelphia, Pennsylvania on June 10-13, 1985, will soon be available to the public. A lay summary of this meeting will also be available.

Dr. Gottesman said a meeting of the RAC Working Group on Viruses will be held on November 12, 1985. The group will discuss containment for retroviruses and viral vectors in response to questions raised by the RAC Risk Assessment Subcommittee. She said she would report to RAC at its next meeting on the meeting of that working group.

XII. ADJOURNMENT

The meeting adjourned at 2:55 p.m. on Monday, September 23, 1985.

Respectfully submitted,

Elizabeth Milewski

Elizabeth A. Milewski, Ph.D.
Rapporteur

William J. Gartland, Jr.

William J. Gartland, Jr., Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

Robert E. Mitchell

1/27/86
Date

Robert E. Mitchell, LL.B.
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SEPTEMBER 1985

NATIONAL INSTITUTES OF HEALTH
POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF
HUMAN SOMATIC-CELL GENE THERAPY PROTOCOLS

WORKING GROUP ON HUMAN GENE THERAPY
NIH RECOMBINANT DNA ADVISORY COMMITTEE

OUTLINE

Applicability

Introduction

I. Description of Proposal

A. Objectives and rationale of the proposed research

B. Research design, anticipated risks and benefits

1. Structure and characteristics of the biological system

2. Preclinical studies, including risk assessment studies

3. Clinical procedures, including patient monitoring

4. Public-health considerations

5. Qualifications of investigators, adequacy of laboratory and clinical facilities

C. Selection of patients

D. Informed consent

E. Privacy and confidentiality

September 23, 1985

II. Special Issues

A. Provision of accurate information to the public

B. Timely communication of research methods and results to investigators and clinicians

III. Requested Documentation

A. Original protocol

B. IRB and IBC minutes and recommendations

C. One-page abstract of gene therapy protocol

D. One-page description of proposed experiment in non-technical language

E. Curricula vitae for professional personnel

F. Indication of other federal agencies to which the protocol is being submitted

G. Other pertinent material

IV. Reporting Requirements

NATIONAL INSTITUTES OF HEALTH

POINTS TO CONSIDER IN THE DESIGN AND SUBMISSION OF HUMAN

SOMATIC-CELL GENE THERAPY PROTOCOLS

Applicability - These "Points to Consider" apply only to research conducted at or sponsored by an institution that receives any support for recombinant DNA research from the National Institutes of Health (NIH). This includes research performed by NIH directly.

Introduction

(1) Experiments in which recombinant DNA[1] is introduced into cells of a human subject with the intent of stably modifying the subject's genome are covered by Section III-A-4 of the NIH Guidelines for Research Involving Recombinant DNA Molecules (49 Federal Register 46266). Section III-A-4 requires such experiments to be reviewed by the NIH Recombinant DNA Advisory Committee (RAC) and approved by the NIH. RAC consideration of each proposal will be on a case-by-case basis and will follow publication of a precis of the proposal in the Federal Register, an opportunity for public comment, and a review of the proposal by the working group of the RAC. RAC recommendations on each proposal will be forwarded to the NIH Director for a decision which will then be published in the Federal Register. In accordance with Section IV-C-1-b of the NIH Guidelines, the NIH Director may approve proposals only if he finds that they present "no significant risk to health or the environment."

(2) In general, it is expected that somatic-cell gene therapy protocols will not present a risk to the environment as the recombinant DNA is expected to be confined to the human subject. Nevertheless, Section I-B-4-b of the "Points to Consider" document asks the researchers to address specifically this point.

[1]Section III-A-4 applies both to recombinant DNA as well as to DNA derived from recombinant DNA.

(3) This document is intended to provide guidance in preparing proposals for NIH consideration under Section III-A-4 of the NIH Guidelines for Research Involving Recombinant DNA Molecules. Not every point mentioned in the "Points to Consider" document will necessarily require attention in every proposal. The document will be considered for revision as experience in evaluating proposals accumulates and as new scientific developments occur. This review will be carried out at least annually.

(4) A proposal will be considered by the RAC only after the protocol has been approved by the local Institutional Biosafety Committee (IBC) and by the local Institutional Review Board (IRB) in accordance with Department of Health and Human Services (DHHS) Regulations for the Protection of Human Subjects (45 Code of Federal Regulations, Part 46). If a proposal involves children, special attention should be paid to subpart D of these DHHS regulations. The IRB and IBC may, at their discretion, condition their approval on further specific deliberation by the RAC and its working group. Consideration of gene therapy proposals by the RAC may proceed simultaneously with review by any other involved federal agencies[2] provided that the RAC is notified of the simultaneous review. Meetings of the committee will be open to the public except where trade secrets or proprietary information would be disclosed. The committee would prefer that the first proposals submitted for RAC review contain no proprietary information or trade secrets, enabling all aspects of the review to be open to the public. The public review of these protocols will serve to inform the

[2]The Food and Drug Administration (FDA) has jurisdiction over drug products intended for use in clinical trials of human somatic-cell gene therapy. For general information on FDA's policies and regulatory requirements, please see the Federal Register, Volume 49, pages 50878-80, 1984.

public not only on the technical aspects of the proposals but also on the meaning and significance of the research.

(5) The clinical application of recombinant DNA techniques to human gene therapy raises two general kinds of questions: (1) the questions usually discussed by IRBs in their review of any proposed research involving human subjects; and (2) broader social issues. The first type of question is addressed principally in Part I of this document. Several of the broader social issues surrounding human gene therapy are discussed later in this Introduction and in Part II below.

(6) Following the Introduction, this document is divided into four parts. Part I deals with the short-term risks and benefits of the proposed research to the patient[3] and to other people, as well as with issues of fairness in the selection of patients, informed consent, and privacy and confidentiality. In Part II, investigators are requested to address special issues pertaining to the free flow of information about clinical trials of gene therapy. These issues lie outside the usual purview of IRBs and reflect general public concerns about biomedical research. Part III summarizes other requested documentation that will assist the RAC and its working group in their review of gene therapy proposals. Part IV specifies reporting requirements.

(7) A distinction should be drawn between making genetic changes in somatic cells and in germ line cells. The purpose of somatic cell gene therapy is to treat an individual patient, e.g., by inserting a properly functioning

[3]The term "patient" and its variants are used in the text as a shorthand designation for "patient-subject."

gene into a patient's bone marrow cells in vitro and then reintroducing the cells into the patient's body. In germ line alterations, a specific attempt is made to introduce genetic changes into the germ (reproductive) cells of an individual, with the aim of changing the set of genes passed on to the individual's offspring. The RAC and its working group will not at present entertain proposals for germ line alterations but will consider for approval protocols involving somatic-cell gene therapy.

(8) The acceptability of human somatic-cell gene therapy has been addressed in several recent public documents as well as in numerous academic studies. The November 1982 report of the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research, Splicing Life, resulted from a two-year process of public deliberations and hearings; upon release of that report, a House subcommittee held three days of public hearings with witnesses from a wide range of fields from the biomedical and social sciences to theology, philosophy, and law. In December 1984, the Office of Technology Assessment released a background paper, Human Gene Therapy, which brought these earlier documents up-to-date. As the latter report concluded:

> "Civic, religious, scientific, and medical groups have all accepted, in principle, the appropriateness of gene therapy of somatic cells in humans for specific genetic diseases. Somatic cell gene therapy is seen as an extension of present methods of therapy that might be preferable to other technologies."

(9) Concurring with this judgment, the RAC and its working group are prepared to consider for approval somatic-cell therapy protocols, provided that the design of such experiments offers adequate assurance that their consequences will not go beyond their purpose, which is the same as the traditional purpose of all clinical investigations, namely, to benefit the health and well-being of the individual being treated while at the same time gathering generalizable knowledge.

(10) Two possible undesirable consequences of somatic-cell therapy would be unintentional (1) vertical transmission of genetic changes from an individual to his or her offspring or (2) horizontal transmission of viral infection to other persons with whom the individual comes in contact. Accordingly, this document requests information that will enable the RAC and its working group to assess the likelihood that the proposed somatic-cell gene therapy will inadvertently affect reproductive cells or lead to infection of other people (e.g., treatment personnel or relatives).

(11) In recognition of the social concern that surrounds the general discussion of human gene therapy, the working group will continue to consider the possible long-range effects of applying knowledge gained from these and related experiments. While research in molecular biology could lead to the development of techniques for germ line intervention or for the use of genetic means to enhance human capabilities rather than to correct defects in patients, the working group does not believe that these effects will follow immediately or inevitably from experiments with somatic-cell gene therapy. The working group will cooperate with other groups in assessing the possible long-term consequences of somatic-cell gene therapy

and related laboratory and animal experiments in order to define appropriate human applications of this emerging technology.

(12) Responses to the questions raised in these "Points to Consider" should be provided in the form of either written answers or references to specific sections of the protocol or its appendices.

I. Description of Proposal

A. Objectives and rationale of the proposed research

State concisely the overall objectives and rationale of the proposed study. Please provide information on the following specific points:

1. Why is the disease selected for treatment by means of gene therapy a good candidate for such treatment?

2. Describe the natural history and range of expression of the disease selected for treatment. In your view, are the usual effects of the disease predictable enough to allow for meaningful assessment of the results of gene therapy?

3. Is the protocol designed to prevent all manifestations of the disease, to halt the progression of the disease after symptoms have begun to appear, or to reverse manifestations of the disease in seriously ill victims?

4. What alternative therapies exist? In what groups of patients are these therapies effective? What are their relative advantages and disadvantages as compared with the proposed gene therapy?

B. Research design, anticipated risks and benefits

1. Structure and characteristics of the biological system

Provide a full description of the methods and reagents to be employed for gene delivery and the rationale for their use. The following are specific points to be addressed:

a. What is the structure of the cloned DNA that will be used?

(1) Describe the gene (genomic or cDNA), the bacterial plasmid or phage vector, and the delivery vector (if any). Provide complete nucleotide sequence analysis or a detailed restriction enzyme map of the total construct.

(2) What regulatory elements does the construct contain (e.g., promoters, enhancers, polyadenylation sites, replication origins, etc.)?

(3) Describe the steps used to derive the DNA construct.

b. What is the structure of the material that will be administered to the patient?

(1) Describe the preparation, structure, and composition of the materials that will be given to the patient or used to treat the patient's cells.

(a) If DNA, what is the purity (both in terms of being a single DNA species and in terms of other contaminants)?

What tests have been used and what is the sensitivity of the tests?

(b) If a virus, how is it prepared from the DNA construct? In what cell is the virus grown (any special features)? What medium and serum are used? How is the virus purified? What is its structure and purity? What steps are being taken (and assays used with their sensitivity) to detect and eliminate any contaminating materials (nucleic acids, proteins, etc.) or contaminating viruses or other organisms in the cells or serum used for preparation of the virus stock?

(2) Describe any other material to be used in preparation of the material to be administered to the patient. For example, if a viral vector is proposed, what is the nature of the helper virus or cell line? If carrier particles are to be used, what is the nature of these?

2. Preclinical studies, including risk-assessment studies

Describe the experimental basis (derived from tests in cultured cells and animals) for claims about the efficacy and safety of the proposed system for gene delivery.

a. Laboratory studies of the delivery system

(1) What cells are the intended recipients of gene therapy? If recipient cells are to be treated in vitro and returned to the patient, how will the cells be characterized before

and after treatment? What is the theoretical and practical basis for assuming that only the treated cells will act as recipients?

(2) Is the delivery system efficient? What percentage of the target cells contain the added DNA?

(3) How is the structure of the added DNA sequences monitored and what is the sensitivity of the analysis? Is the added DNA extrachromosomal or integrated? Is the added DNA unrearranged?

(4) How many copies are present per cell? How stable is the added DNA both in terms of its continued presence and its structural stability?

b. Laboratory studies of gene expression

Is the added gene expressed? To what extent is expression only from the desired gene (and not from the surrounding DNA)? In what percentage of cells does expression from added DNA occur? Is the product biologically active? What percentage of normal activity results from the inserted gene? Is the gene expressed in cells other than the target cells? If so, to what extent?

c. Laboratory studies pertaining to the safety of the delivery/expression system

(1) If a retroviral system is used:

(a) What cell types have been infected with the retroviral vector preparation? Which cells, if any, produce infectious particles?

(b) How stable are the retroviral vector and the resulting provirus against loss, rearrangement, recombination, or mutation? What information is available on how much rearrangement or recombination with endogenous or other viral sequences is likely to occur in the patient's cells? What steps have been taken in designing the vector to minimize instability or variation? What laboratory studies have been performed to check for stability, and what is the sensitivity of the analyses?

(c) What laboratory evidence is available concerning potential harmful effects of the treatment, e.g., development of neoplasia, harmful mutations, regeneration of infectious particles, or immune responses? What steps have been taken in designing the vector to minimize pathogenicity? What laboratory studies have been performed to check for pathogenicity, and what is the sensitivity of the analyses?

(d) Is there evidence from animal studies that vector DNA has entered untreated cells, particularly germ line cells? What is the sensitivity of the analyses?

(e) Has a protocol similar to the one proposed for a clinical trial been carried out in non-human primates and/or other animals? What were the results? Specifically, is there any evidence that the retroviral vector has recombined with any endogenous or other viral sequences in the animals?

(2) If a non-retroviral delivery system is used: What animal studies have been done to determine if there are pathological or other undesirable consequences of the protocol (including insertion of DNA into cells other than those treated, particularly germ line cells)? How long have the animals been studied after treatment? What tests have been used and what is their sensitivity?

3. Clinical procedures, including patient monitoring

Describe the treatment that will be administered to patients and the diagnostic methods that will be used to monitor the success or failure of the treatment. If previous clinical studies using similar methods have been performed by yourself or others, indicate their relevance to the proposed study.

a. Will cells (e.g., bone marrow cells) be removed from patients and treated in vitro in preparation for gene therapy? If so, what kinds of cells will be removed from the patients, how many, how often, and at what intervals?

b. Will patients be treated to eliminate or reduce the number of cells containing malfunctioning genes (e.g., through radiation or chemotherapy) prior to gene therapy?

c. What treated cells (or vector/DNA combination) will be given to patients in the attempt to administer gene therapy? How will the treated cells be administered? What volume of cells will be used? Will there be single or multiple treatments? If so, over what period of time?

d. What are the clinical endpoints of the study? How will patients be monitored to assess specific effects of the treatment on the disease? What is the sensitivity of the analyses? How frequently will follow-up studies be done? How long will patient follow-up continue?

e. What are the major potential beneficial and adverse effects of treatment that you anticipate? What measures will be taken in an attempt to control or reverse these adverse effects if they occur? Compare the probability and magnitude of potential adverse effects on patients with the probability and magnitude of deleterious consequences from the disease if gene therapy is not performed.

f. If a treated patient dies, what special studies will be performed as part of the autopsy?

4. Public-health considerations

Describe any potential benefits and hazards of the proposed therapy to persons other than the patients being treated. Specifically:

a. On what basis are potential public health benefits or hazards postulated?

b. Is there a significant likelihood that the added DNA will spread from the patient to other persons or to the environment?

c. Will any precautions be taken against such spread (e.g., to patients sharing a room, health-care workers, or family members)?

d. What measures will be undertaken to mitigate the risks, if any, to public health?

5. Qualifications of investigators, adequacy of laboratory and clinical facilities

Indicate the relevant training and experience of the personnel who will be involved in the preclinical studies and clinical administration of gene therapy. In addition, please describe the laboratory and clinical facilities where the proposed study will be performed.

a. What professional personnel (medical and nonmedical) will be involved in the proposed study? What are their specific qualifications and experience with respect to the disease to be treated and with respect to the techniques employed in molecular biology? Please provide curricula vitae (see Section III-E).

b. At what hospital or clinic will the treatment be given? Which facilities of the hospital or clinic will be especially important for the proposed study? Will patients occupy regular hospital beds or clinical research center beds? Where will patients reside during the follow-up period?

C. Selection of patients

Estimate the number of patients to be involved in the proposed study of gene therapy. Describe recruitment procedures and patient eligibility requirements, paying particular attention to whether these procedures and requirements are fair and equitable.

1. How many patients do you plan to involve in the proposed study?

2. How many eligible patients do you anticipate being able to identify each year?

3. What recruitment procedures do you plan to use?

4. What selection criteria do you plan to employ? What are the exclusion and inclusion criteria for the study?

5. How will patients be selected if it is not possible to include all who desire to participate?

D. Informed consent

Indicate how patients will be informed about the proposed study and how their consent will be solicited. The consent procedure should adhere to the requirements of DHHS regulations for the protection of human subjects (45 Code of Federal Regulations, Part 46). If the study

involves pediatric or mentally handicapped patients, describe procedures for seeking the permission of parents or guardians and, where applicable, the assent of each patient. Areas of special concern highlighted below include potential adverse effects, financial costs, privacy, and long-term follow-up.

1. How will the major points covered in Sections I-A through I-C of this document be disclosed to potential participants in this study and/or parents or guardians in language that is understandable to them?

2. How will the innovative character and the theoretically-possible adverse effects of gene therapy be discussed with patients and/or parents or guardians? How will the potential adverse effects be compared with the consequences of the disease? What will be said to convey that some of these adverse effects, if they occur, could be irreversible?

3. What explanation of the financial costs of gene therapy and any available alternative therapies will be provided to patients and/or parents or guardians?

4. How will patients and/or their parents or guardians be informed that the innovative character of gene therapy may lead to great interest by the media in the research and in treated patients?

5. How will patients and/or their parents or guardians be informed:

   a. That some of the procedures performed in the study may be irreversible?

   b. That following the performance of such procedures it would not be medically advisable for patients to withdraw from the study?

   c. That a willingness to cooperate in long-term follow-up (for at least three to five years) will be a precondition for participation in the study?

   d. That a willingness to permit an autopsy to be performed in the event of a patient's death following treatment is also a precondition for a patient's participation in the study? (This stipulation is included because an accurate determination of the precise cause of a patient's death would be of vital importance to all future gene therapy patients.)

E. Privacy and confidentiality

Indicate what measures will be taken to protect the privacy of gene therapy patients and their families as well as to maintain the confidentiality of research data.

1. What provisions will be made to honor the wishes of individual patients (and the parents or guardians of pediatric or mentally handicapped patients) as to whether, when, or how the identity of patients is publicly disclosed?

2. What provision will be made to maintain the confidentiality of research data, at least in cases where data could be linked to individual patients?

II. Special Issues

Although the following issues are beyond the normal purview of local IRBs, the RAC and its working group request that investigators respond to questions A and B below.

A. What steps will be taken, consistent with point I-E above, to ensure that accurate information is made available to the public with respect to such public concerns as may arise from the proposed study?

B. Do you or your funding sources intend to protect under patent or trade secret laws either the products or the procedures developed in the proposed study? If so, what steps will be taken to permit as full communication as possible among investigators and clinicians concerning research methods and results?

III. Requested Documentation

In addition to responses to the questions raised in these "Points to Consider," please submit the following materials:

A. Your protocol as approved by your local IRB and IBC. The consent form, which must have IRB approval, should be submitted to the NIH only on request.

B. Local IRB and IBC minutes and recommendations that pertain to your protocol.

C. A one-page scientific abstract of the gene therapy protocol.

D. A one-page description of the proposed experiment in nontechnical language.

E. Curricula vitae for professional personnel.

F. An indication of other federal agencies to which the protocol is being submitted for review.

G. Any other material which you believe will aid in the review.

IV. Reporting Requirements

A. Serious adverse effects of treatment should be reported immediately to both your local IRB and the NIH Office for Protection from Research Risks, and a written report should be filed with both groups. A copy of the report should also be forwarded to the NIH Office of Recombinant DNA Activities (ORDA).

B. Reports regarding the general progress of patients should be filed at six-month intervals with both your local IRB and ORDA. These twice-yearly reports should continue for a sufficient period of time to allow observation of all major effects (at least three to five years). In the event of a patient's death, the autopsy report should be submitted to the IRB and ORDA.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE
WORKING GROUP ON VIRUSES
MINUTES OF MEETING[1]

NOVEMBER 12, 1985

The Working Group on Viruses of the Recombinant DNA Advisory Committee was convened at 9:00 a.m. on November 12, 1985, at the National Institutes of Health, Building 16, Stone House, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. Susan Gottesman was the Chair. The following people were present for all or part of the meeting:

Working Group members:

W. French Anderson
David Friedman
Susan Gottesman
Terri Grodzicker
Janet Hartley
Wolfgang Joklik
Arthur Landy
Robert Martin
Richard Mulligan
Fred Rapp
Robert Simpson
Stanley Barban
(Executive Secretary)

A working group roster is attached (Attachment I).

Other National Institutes of Health staff:

Barrie Carter, NIADDKD
Elizabeth Milewski, NIAID

Others:

Neal First, University of Wisconsin
Carol Gronbeck, Genentech, Inc.
Lynn Harding, Harvard University
Dan Liberman, Massachusetts Institute of Technology
Ralph Smalling, Amgen
LeRoy Walters, Kennedy Institute, Georgetown University

[1]The working group is advisory to the RAC, and its recommendations should not be considered as final or accepted.

Dr. Gottesman convened the meeting of the Working Group on Viruses at 9:00 a.m. on November 12, 1985. She said this working group was formed to: (1) address the impact of recent discoveries in virology on the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules; and (2) respond to scientific issues originating in a memorandum from the Department of Health and Human Services (DHHS).

In April 1984, the Assistant Secretary for Planning and Evaluation, DHHS, sent a memorandum to the Assistant Secretary for Health, DHHS. This memorandum transmitted to the Assistant Secretary for Health a list of issues in recombinant DNA technology. The Assistant Secretary for Health forwarded this memorandum for comment to several DHHS agencies: the Food and Drug Administration (FDA), the Centers for Disease Control (CDC), and the National Institutes of Health (NIH). The Director, NIH, subsequently asked the Recombinant DNA Advisory Committee (RAC) Risk Assessment Subcommittee to formulate a response to the memorandum. The Risk Assessment Subcommittee upon evaluation of the memorandum suggested some issues involving use of viral vectors should be evaluated in greater detail by a working group of virologists.

Dr. Gottesman said containment conditions and review procedures for experiments involving viral genes are specified in several sections of the NIH Guidelines: (1) Under Appendix C in certain conditions, viral genes may be cloned in E. coli, S. cerevisiae, and B. subtilis host-vector systems. These experiments are exempt. (2) Under Section III-B, viruses classified in Appendix B can be used under specified containment which ranges from Biosafety Level (BL) 2 to BL3. The Institutional Biosafety Committee (IBC) must approve of the experiments before initiation. Many retroviruses have not, however, been classified in Appendix B although some have been listed in this appendix. (3) Under Section III-C, Experiments That Require IBC Notice Simultaneously With Initiation of Experiments, experiments involving viruses are also covered. Section III-C contains a caution which reads as follows:

> "Experiments Involving Formation of Recombinant DNA Molecules Containing No More Than Two-Thirds of the Genome of Any Eukaryotic Virus. Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus (all viruses from a single Family (17) being considered identical (19)) may be propagated and maintained in cells in tissue culture using BL1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Family of defective viruses being used. If helper virus is present, procedures specified under Section III-B-3 should be used. The DNA may contain fragments of the genome of viruses from more than one Family but each fragment must be less than two-thirds of a genome."

Dr. Gottesman said the NIH Guidelines define a defective virus as consisting of less than two-thirds of a virus genome.

Dr. Gottesman said the working group would address several specific issues at this meeting. One issue is whether Appendix B should be revised to reflect more accurately the situation with retroviruses. A second issue is whether the NIH Guidelines should be modified to cover explicitly recombinant RNA.

The NIH Guidelines currently refer only to recombinant DNA and do not refer to recombinant RNA although RNA viruses have been covered under case law. She said a RAC subworking group had developed a proposal to modify the NIH Guidelines to cover explicitly recombinant RNA (Attachment II), and the Working Group on Viruses would evaluate this proposal.

Dr. Gottesman asked Dr. Simpson, the Chair of the NIH IBC, to describe the issues he wished the working group to address.

Dr. Simpson said the NIH Office of Recombinant DNA Activities (ORDA) has been informing investigators that experiments involving human oncogenes should be performed under BL2 containment. ORDA does not address, however, experiments involving oncogenes from other animal sources. He suggested the working group address the issue of how oncogenes should be handled under the NIH Guidelines.

Dr. Simpson also suggested the working group consider whether experiments involving single viral genes, e.g., the Herpes simplex thymidine kinase (TK) gene, in tissue culture should be registered with IBCs or whether such experiments might be exempted from the NIH Guidelines under Appendix C, Exemptions Under Section III-D-5.

Dr. Dan Liberman of the Massachusetts Institute of Technology (MIT) asked the working group to reevaluate the use of viral vectors with viral gene inserts in light of recent developments.

Dr. Gottesman asked the working group to begin by addressing the proposal (Attachment II) to modify the NIH Guidelines to specifically refer to recombinant RNA. She asked the working group whether this proposal would adequately address the use of RNA viruses under the NIH Guidelines. The working group agreed the proposal (Attachment II) was appropriate.

Dr. Gottesman asked the working group to next address the issue of whether experiments involving single viral genes in tissue culture should be exempt from the NIH Guidelines. She said under Appendix C, experiments involving the cloning of fragments of less than two-thirds of a virus in an E. coli K-12 host-vector system are exempt; however, if a viral gene is introduced into eukaryotic cells in tissue culture, the experiments must be registered with the IBC. She did not think tissue culture experiments involving single genes of viral origin need to be registered with the IBC. Dr. Grodzicker agreed.

Dr. Joklik said two issues must be considered in evaluating experiments involving viruses: (1) the intrinsic infectivity of the virus; and (2) the rescuability of the virus by helper virus. The working group could address viral intrinsic infectivity by limiting the fraction of the genome that can be introduced and propagated in tissue culture systems.

Dr. Gottesman said the working group might suggest the NIH Guidelines be modified so as not to require IBC registration of tissue culture experiments involving less than one-third of a virus of a single Family.

Dr. Joklik felt the NIH Guidelines should specify less than two-thirds of a viral genome as a defective virus; he felt this specification a reasonable limit for requiring IBC registration of experiments in tissue culture. He thought specifying a limit of less than one-third of a viral genome could create problems; for example, the Rhabdovirus L gene accounts for more than 50% of the total Rhabdovirus genome. This gene could not be cloned without registrating the project with the IBC if a limit of one-third of the viral genome was specified.

Dr. Martin said designating as defective some fractional portion of a genome, e.g., less than two-thirds of a virus genome, is artificial. Herpes viruses, for example, can almost grow in tissue culture with one-third of the genome deleted. Dr. Joklik agreed a large portion of the genome of certain viruses can be deleted, and the viruses will still replicate in certain cells in culture. Dr. Rapp added there is no evidence such a truncated virus could replicate in humans or other hosts.

Dr. Gottesman asked the working group to discuss the possibility of rescue of defective viral genomes through recombination.

Dr. Anderson said the presence of helper virus or endogenous viral sequences in a tissue culture system should be considered with retroviral systems. Two-thirds of the Moloney Murine Leukemia virus (MoMLV) genome could recombine with helper virus to create an infectious retrovirus depending on the cell type used to culture the defective genome.

[Rapporteur's Note: Retroviruses are animal viruses with a glycoprotein envelope and an RNA genome that replicates through a DNA intermediate. The DNA intermediate, the provirus, is stably integrated into cellular DNA.

There are a number of ways in which retroviral genomes can acquire new genetic information. These include reverse transcription catalyzed recombination events (which necessitate the co-encapsidation of different RNA species) as well as recombinational events involving endogenous viral sequences.]

Dr. Mulligan pointed out that in some cases very small regions of a retroviral genome can be acquired by defective genomes which restore infectivity. For instance, cell lines have been constructed which permit the encapsidation of defective recombinant genomes. These cell lines contain a proviral genome which gives rise to all the viral proteins necessary for the encapsidation process, yet lacks the sequnce necessary for the encapsidation of its own genomic RNA. With some of these cell lines, transfection of constructs containing an intact "packaging" sequence can lead to recombinational events reslting in the generation of fully infectious viruses. Dr. Anderson said this type of rescue would depend on the cells used to grow the defective retrovirus.

Dr. Mulligan said the recombination frequency of retroviruses is approximately 10^{-5} under optimal conditions. A lower frequency is observed when retroviruses recombine with endogenous viral sequences in the cell; recombination frequency then depends on the abundance of endogenous complementing sequences.

Dr. Landy asked whether retroviruses carry gratuitous genes, i.e., genes non-essential for virus replication. Dr. Mulligan replied they could, but these constructs are usually defective viruses. Retroviruses from chickens and a specialized class of retroviruses are exceptions. Rous sarcoma virus, for example, is replication competent yet carries in addition the src oncogene. The Human T-Lymphotropic Virus (HTLV) class of retroviruses carry a novel gene in addition to the standard viral gag, pol and env genes, although it is unlikely this gene is "gratuitous." In general, however, even though recombination occurs at high frequency in retroviruses, it is not likely the resulting virus would contain gratuitous genetic information because genes in the coding region of retroviruses cannot be easily substituted and infectious particles still produced.

[Rapporteur's Note: There appears to be a maximum limit on the size of the RNA in infectious retrovirus virions. The molecular basis for this size limit is unknown. It could be related to problems of packaging viral RNA in the isocahedral core or to problems in reverse transcription or both. Typically in constructing a vector, the gene of interest replaces essential virion genes and renders virus replication defective. Added genes can be readily eliminated by spontaneous deletion. Some replication-defective, larger than normal constructs, are possible.

In the special case of the Rous sarcoma virus, the virus adds the oncogenic src gene to the essential virion genes without disrupting vital functions. In all other known cases, the inclusion of cellularly-derived transforming sequences is associated with loss of some or all of the coding regions found in replication-competent retroviruses. Thus, these modified viruses are replication-defective. Such viruses are generated at a very low frequency in nature.]

Dr. Landy asked whether host cells can provide helper functions for retroviruses carrying foreign genes or if the helper function must also be transferred. Dr. Mulligan said specialized cell lines have been constructed that can provide all the viral functions necessary for encapsidation and transmission of recombinant genomes. The virus produced in these cell lines can only go through one replication cycle. The modified retrovirus might continue through a second round of replication in other cells if it can encounter and infect cells which possess the necessary helper function.

Dr. Martin asked if DNA viruses such as SV40 can be rescued in tissue culture systems. Dr. Joklik replied they can be rescued but at very low frequencies.

Dr. Gottesman asked whether recombinants from tissue culture experiments could pose a danger to laboratory workers even though the possibility of rescue is low in experiments involving one-third of a viral genome. Would the virus infect human cells? Could the virus recombine with endogenous sequences and would the resulting virus be pathogenic?

Dr. Anderson thought some concern might exist for work with human cell lines. He did not think laboratory personnel would be endangered by work with cell lines other than human cell lines.

Dr. Gottesman asked whether the working group would support a recommendation to exempt experiments in tissue culture involving less than one-third of a viral genome of a specific viral Family.

Dr. Joklik preferred that experiments involving less than one-half of the genome of a specific viral Family be exempted from the NIH Guidelines.

Dr. Mulligan said he did not see a great deal of difference between exempting one-third and one-half of the genome. He preferred the working group recommend experiments involving less than one-half of a viral genome be exempted from the NIH Guidelines.

Dr. Anderson thought the working group should suggest experiments involving less than one-half of nonprimate virus genomes and less than one-third of the genome of primate viruses be exempted from the NIH Guidelines. Dr. Joklik agreed primate retroviruses may warrant special evaluation in terms of rescue in tissue culture systems.

Dr. Gottesman asked how large viruses, such as Herpes viruses, should be handled under such a proposal.

Dr. Mulligan said language referring to two-thirds of a genome would apply primarily to smaller viruses; it is not possible to clone two-thirds of large viruses such as the Herpes viruses.

Dr. Mulligan suggested Herpes viridae and other large viruses are a special case which might be distinguished in any language developed as part of a recommendation for modifying the NIH Guidelines. Dr. Rapp agreed; he reasoned Herpes viruses deleted for one-half of the genome might be viable in tissue culture since Herpes viruses range in size from 70×10^6 to 150×10^6 daltons.

Dr. Anderson suggested the working group might specifically develop language to apply to virus fragments smaller than 50 kilobases.

Dr. Gottesman said she preferred simple language and thought a strong argument for exempting one-third of a viral genome in tissue culture systems was that few mechanisms exist for spread of these small pieces of viruses.

Dr. Landy said a modification exempting tissue culture experiments involving less than one-third of a viral genome of a given virus Family would reduce the IBC workload enormously and would achieve the working group goal of eliminating the requirement to register experiments involving single viral genes.

Dr. Simpson said he would attempt to develop with the advice and assistance of the NIH IBC a proposal to modify the NIH Guidelines. He would request that RAC consider this proposal. The working group agreed.

Dr. Gottesman asked the working group to discuss the issue of exempting single viral genes in tissue culture experiments while distinguishing between the protein product of oncogenes and the products of genes such as the TK gene.

[Rapporteur's Note: Oncogenes were first identified as the transforming (onc) genes of retroviruses. Typically, these viruses initiate and maintain cancers with autonomous onc genes that are dominant in susceptible cells. The viral onc genes are not essential to the multiplication of the virus. The oncogenic retroviruses are the products of recombinational events between retroviruses and normal cellular genes. The normal cellular genes related to transforming retroviral onc genes are termed "proto-onc" genes. The relationship between viral onc genes and proto-onc genes shows the proto-onc genes to be genes which normally produce products necessary to cellular growth which if subjected to abnormal regulation can be involved in carcinogenesis. Some evidence indicates that activated proto-onc genes are involved in carcinogenesis.]

Dr. Gottesman asked the working group to construct a "worst case" analysis and to consider potential effects of oncogene products if these products are expressed by a bacterial host-vector system which can colonize the human gastrointestinal (GI) tract. Dr. Gottesman said in a "worst case" hypothetical analysis, one would assume that an E. coli host-vector system expressing high levels of the product had colonized a human GI tract. One would also assume that a high level expressing strain would produce approximately 10^6 molecules of oncogene product per cell per generation (about 30 percent of the possible protein initiations). Another assumption in a "worst case" calculation is that a human GI tract completely colonized by the engineered bacterial strain would contain 2×10^9 engineered E. coli duplicating about every 12 hours. Assuming such an event, is there any mechanism by which the product could affect the cells of the gut? Is there any basis for believing the bacteria could proliferate and deliver the oncogene product to target cells in the GI tract?

Dr. Grodzicker said the normal function of the proto-oncogenes is poorly understood. On the basis of in vitro assays one product appears to be a growth factor, another is a membrane protein growth factor receptor, others probably have different functions. Two of these genes resemble genes of the yeast cell cycle. Dr. Grodzicker said the effect of some oncogene products (e.g., the ras gene, SV40T, the src gene) has been tested in vitro in tissue culture. A short term effect is seen when cells are exposed to some oncogene products, but this effect disappears quickly when the oncogene products are removed.

Dr. Joklik said he knew of no reports of irreversible effects from exposure of tissue culture cells to oncogene products. Dr. Rapp said the gene is oncogenic not the gene product.

Dr. Landy said the important issue is that recombinant DNA technology can produce large amounts of viral products that could not be obtained with the virus itself. He asked if in addition to GI exposure there were other routes of exposure which should be considered such as systemic infection or aerosol exposure. He asked whether exposure to large amounts of an oncogene product could affect an individual. Dr. Rapp said large amounts of any natural substance introduced into a subject will have an effect. The question is whether there is a route of entry for such a large amount of substance.

Dr. Landy said some of the cells in the GI tract may have receptors for growth factors. Could the presence of large amounts of growth factor in the GI tract result in tumor formation? Are growth factors highly biologically active molecules? Would exposure to small amounts have an effect?

Dr. Martin conjectured that an animal exposed to large amounts of a potent growth factor secreted by an E. coli host-vector system could develop a tumor of the GI tract. He did not know if the tumor would recede when the growth factor was no longer present. He did not think the tumor would continue to grow in the absence of growth factor. He pointed out, however, that the effect of growth factor on cells in culture cannot be compared with the in vivo situation; the normal intestine continually grows but growth factors are assayed by their stimulatory effect on stationary phase tissue culture cells.

Dr. Anderson agreed. He said fibroblasts in tissue culture may possess receptors for specific growth factors; however, the intestinal mucosa is designed to absorb nutrients and may not possess receptors for growth factors.

Dr. Simpson did not think enteral exposure to an oncogene product would be of great concern. He would be more concerned with parenteral exposure. He cited the case of Shiga toxin which is very potent when introduced parenterally in small amounts but has no effect on monkeys when large amounts are introduced into intestinal pouches.

[Rapporteur's Note: In 1953, Branham, Dack, and Riggs showed that large amounts of Shiga toxin instilled directly into monkey intestinal pouches has no effect. These investigators administered to monkey intestinal pouches with no observed effect the equivalent of approximately 20,000 lethal doses administered parenterally. In a worst case scenario, 10^9 engineered E. coli with the Shiga toxin gene on a high expression, high copy number plasmid might produce one milligram of toxin in the human gut. This amount is roughly equivalent to approximately 14,000 lethal doses for humans when the toxin is administered parenterally.]

Dr. Gottesman asked whether any mechanism exists by which a retrovirus cloned in E. coli host-vector systems could become an infectious particle. She asked whether the retroviral transformation system could function in prokaryotic systems and whether prokaryotic systems could enhance retroviral replication.

[Rapporteur's Note: One of the initial concerns regarding the use of E. coli host-vector systems to molecularly clone animal virus genomes was that the bacteria might spread epidemically through the ecosystem and deliver potentially infectious virus particles/virus genomes to tissues not ordinarily exposed to such agents. To address some of these concerns, Chan et al., and Israel et al., conducted experiments to determine whether E. coli K-12 harboring recombinant phage or plasmids (such as pBR322) containing the polyoma virus genome (PY) could cause a spreading infection in sensitive test animals (mice). In the experiments, genetically disabled E. coli K-12 containing PY-pBR322 recombinant plasmids was fed or inoculated into mice. None of the animals developed a PY

virus infection as monitored by the development of hemagglutination-inhibiting antibodies.

These experiments were subsequently extended (Israel et al.) to evaluate whether the same PY plasmid-containing E. coli χ1776 strains were tumorigenic in newborn hamsters. In newborn hamsters, inoculation of PY virions induces tumors in 100% of the animals. In control experiments, purified PY virus DNA as well as recombinant plasmids containing PY DNA cause tumors in 5-19% of the inoculated hamsters. The tumorigenicity of PY DNA is at least 10^6-fold lower than that observed for intact PY virus particles. In studies to assess whether bacteria could deliver potentially tumorigenic viral DNA to susceptible cells in an animal model system, subcutaneous inoculation of very large doses (greater than 10^7 organisms/animal) of E. coli K-12 strain χ 1776 containing PY-plasmid recombinants failed to induce tumors. Thus, the results obtained from both animal model systems indicate that potentially infectious or tumorigenic recombinant DNA molecules were not transferred out of the χ 1776 strain of E. coli K-12 into mammalian cells.

To more rigorously evaluate this question, the GI tract of conventional antibiotic-compromised or germ-free mice were colonized with wild-type E. coli harboring various PY-plasmid DNAs (Smith et al.). Although colonization and prolonged excretion of large amounts of E. coli could be readily demonstrated, no evidence of PY virus infection was detected.]

Dr. Martin thought it less likely that an infectious RNA virus would be regenerated in E. coli than an infectious DNA virus since E. coli host-vector systems are not likely to possess the machinery needed to produce an RNA particle from a DNA template. Dr. Grodzicker said the retroviruses evolved to replicate in eukaryotic cells. She did not think they would function in prokaryotic cells.

Dr. Landy asked whether the DNA provirus form of retroviruses would be more infectious than the retroviral particle.

Dr. Mulligan said provirus DNA can transfect eukaryotic cells which do not possess the appropriate retrovirus receptor site and are otherwise nonpermissible. Upon transfection, viral particles can in some cases be produced. The provirus would probably have a stable transfection frequency equivalent to the transfection rate of naked DNA which is approximately 10^{-4}.

Dr. Carol Lax Gronbeck of Genentech, Inc., asked the working group to consider whether workers exposed to a host-vector system expressing retroviral sequences could develop an antibody response to products of these sequences. She added that no Genentech worker has ever converted to seropositive from product exposure. She said this is not surprising since very low levels of worker exposure occur under the BL1-Large-Scale (LS) containment conditions employed by Genentech, Inc.

Dr. Rapp said it is unlikely a worker exposed to host-vector systems carrying a virus but not injected with the virus itself would develop antiviral antibodies. He said this brought up the issue, however, of whether exposure to low levels

of a viral antigen could change the course of the disease caused by that virus. Cross reactivity might also affect the course of a disease; e.g., antibody to Herpes simplex I virus affects the response of patients to Herpes simplex II virus.

Dr. Joklik said low levels of antibodies modify patient responses to infection. In some cases, low levels of antibody are beneficial; in other cases, they are detrimental to the course of the disease. He thought the impact of cross reactivity on the course of the disease would also depend on the virus.

Dr. Simpson suggested data exist in the fermentation industry on worker response to protein exposure. Some of this information might be useful in determining how individuals respond to protein exposure.

Dr. Rapp did not think it would be fruitful to review data on immune response to proteins since proteins differ in ability to elicit responses. Response to exposure to infectious particles differs significantly from exposure to proteins; infectious particles also differ from each other in ability to elicit an immune response.

Dr. Gottesman thought the working group should next discuss how retroviruses should be treated under the NIH Guidelines.

Dr. Anderson thought the NIH Guidelines as currently written are inadequate in dealing with four topics: (1) the retroviral vectors used in human gene therapy; (2) the HTLVs; (3) oncogenes in amphotropic vectors; and (4) the consequences of packaging retroviruses in amphotropic coats. He noted that the NIH Guidelines do not address the impact of "packaging lines" since these cell lines did not exist when the NIH Guidelines were originally written. Dr. Rapp suggested simian retroviruses should also be evaluated.

Dr. Joklik suggested it might be useful to evaluate these issues in relation to procedures investigators are likely to use in the near future; e.g., the vectors used in human gene therapy.

Dr. Gottesman asked the working group to begin with a discussion of the life cycle and host range of retroviruses. She asked whether retroviral host range related to transformation and tissue specificity.

[Rapporteur's Note: Adsorption of the retrovirus to the cell is followed by introduction of the single-stranded RNA genome into the cytoplasm where it is reverse transcribed into a double-stranded DNA molecule containing two long direct repeats, termed long terminal repeats (LTRs), at the ends. Following transport to the nucleus, the circularized molecule integrates into the host chromosome with the LTRs at the junctions between viral and cellular genomes. The integrated provirus serves as the template for synthesis of genomic and subgenomic mRNAs encoding the gag, pol, and env functions as well as full-length transcripts of the viral genome. The full-length transcripts are packaged into virions which are shed from the cell as infectious virus.

In retroviruses, there are a number of virally encoded proteins essential for the replicative process that can be provided in trans by a helper virus. These proteins are coded for by three retrovirus genes found in all replication-competent viruses, regardless of their species of origin. These genes, the gag, pol, and env genes, code for proteins involved respectively in: virion encapsidation and assembly, reverse transcription of viral DNA and integration, and virion adsorption/penetration.

Sequences essential in cis for viral gene expression are: the LTRs which include regulatory sequences for viral RNA synthesis as well as integration sites for insertion of the virus into chromosomal DNA (attachment sequences); the sites involved in the initiation of plus and minus strand reverse transcription; and the primer binding sites (PBS and PPT) utilized in reverse transcription.

Although much has been learned about the regulatory features of retroviruses, uncertainties remain. Those features of the proviral structure that are thought to be necessary for transcription and transmission of the viral genome are: the LTR sequence on each end containing regulatory signals for initiating and terminating transcription, sequences required for reverse transcription and others for proviral integration; short sequences immediately adjacent to each LTR and necessary for reverse transcription; the packaging sequence necessary for the viral RNA to be packaged into an infectious viral particle; and the donor and acceptor splice sites.

Retroviruses have been isolated from many different classes of vertebrates. However, most clases of retroviruses have a fairly limited host range. The most common group of mouse retroviruses, ecotropic murine leukemia viruses (MLV), replicates well only in mouse and rat cells; and the most common species of avian retroviruses, avian leukosis viruses, replicates only in chicken and quail cells. But this limited host range is not characteristic of all retroviruses. Another group of mouse retroviruses, amphotropic murine leukemia viruses, replicates well in cells from several other mammalian species as well as mouse, and another species of avian retroviruses, reticuloendotheliosis virus (Rev), replicates well in all avian cells tested and in dog cells.

The primary determinant of retrovirus host range appears to be the envelope glycoproteins. For example, there are several subgroups of avian leukosis viruses with different host ranges on chicken cells of different genotypes. These avian leukosis virus subgroups differ only in their env gene, the gene that codes for the virion glycoproteins. Similarly, there are at least three subgroups of mouse leukemia viruses, eco-, xeno-, and amphotropic with different host ranges on mammalian and avian cells corresponding to their different env genes.

Other controls on retrovirus host range exist like the effect of the Fv-1 gene on mouse leukemia virus integration and the lack of processing of Rous

sarcoma virus proteins in mammalian cells. In some viruses there are tissue-specific controls on transcriptional activity.]

Dr. Mulligan said the most important factors in determining host range are receptor site recognition, attachment, and penetration. The viral coat protein plays the major role in receptor recognition and on host range. He said some post-penetration restriction of replication occurs in some cell types. However, post-penetration restriction does not have a major impact on host range. In addition, if transcriptional specificity is changed, the tropism of the virus may change; for example, a retrovirus will preferentially replicate in T cells if the retrovirus has that transcriptional specificity.

Dr. Mulligan said a range of manipulations, such as modification of virally encoded envelope proteins, can affect host range. Dr. Mulligan said this "pseudotyping" or "phenotypic mixing" affects the host range for one generation unless the modified protein is an envelope protein encoded by the virus.

[Rapporteur's Note: Encapsidated RNA is budded from the plasma membrance at locations where the membrane is modified by insertion of viral proteins and an exclusion of host cell proteins. Virion maturation involving proteolytic cleavage of certain proteins and changes in morphology of the internal virion core follows budding.

The process of budding is not virus species-specific; the envelope glycoproteins need not be from the retrovirus family. In addition, encapsidation or packaging is not completely species specific although it is more specific than budding. Thus, proteins in a retroviral virion can be encoded by the genome in the virion (replication-competent retrovirus), by a complementing viral genome (helper replication-competent retrovirus or superinfecting retrovirus), or by a helper cell (cell with viral genomes deleted of encapsidation sequences and constitutively expressing virion proteins.)

The envelope-specific control of host range can be abrogated for one virus cycle by fusion of virions to resistant cells using inactivated Sendai virus or polyethylene glycol.

A virus composed of a genome from one retrovirus and envelope proteins from another virus is termed a "pseudotype." A pseudotype represents pseudo-phenotypic mixing; upon infection the genome of the pseudotype will only express genome proteins and progeny particles will be encapsidated by these genomic proteins.]

Dr. Anderson said ecotropic viruses (viruses isolated from mouse cells and only infecting cells of the same species) do not present a hazard to humans since their host range is limited. Viruses with amphotropic coats should on the other hand be evaluated; the NIH Guidelines might address this situation.

Dr. Landy asked what determines which proteins the virus incorporates in its coat.

Dr. Mulligan said the basis for the selection of specific membrane proteins for inclusion in a retrovirus particle is not clear, although the viral gag proteins presumably play some role in the process.

Dr. Joklik said human cells are not thought to produce envelope proteins. Endogenous sequences for such a protein have been identified in the human genome but do not appear to be expressed normally.

Dr. Gottesman asked Dr. Hartley whether murine cells express membrane proteins which are incorporated into retroviral coats. Dr. Hartley said some mouse cell lines express proteins which could be incorporated into retroviral coats.

Dr. Gottesman asked whether there are pseudotypic combinations of retroviral genomes with amphotropic envelope proteins which might present a hazard; e.g., amphotropically packaged retroviruses carrying an oncogene.

Dr. Simpson said some work had been done with mice which suggests that one round of replication of a retrovirus carrying an oncogene is sufficient to cause tumors.

Dr. Liberman said the MIT Biosafety Office is not concerned that an amphotropically packaged replication defective retrovirus might cause an epidemic; the office is concerned, however, that amphotropic retrovirus vectors carrying an oncogene may present a risk of carcinogenesis to laboratory personnel. MIT requires BL3 containment for the first two or three rounds of replication of these agents in order to protect personnel. Containment may be lowered to BL2 for subsequent rounds of replication. Dr. Mulligan said his laboratory uses BL3 containment for research involving amphotropic retroviral vectors carrying an oncogene.

Dr. Gottesman noted that the situation of laboratory workers differs from the situation of the general public in that laboratory personnel would be exposed to the initial rounds of replication of these agents while the public would not be expected to be exposed to these initial rounds.

Dr. Gottesman asked whether special techniques were used in the laboratory in handling the murine retroviruses. Dr. Hartley replied that the good laboratory techniques specified for working with infectious agents are sufficient; under those conditions, there is no evidence of infection of laboratory workers.

Dr. Mulligan asked whether researchers employed special precautions in handling feline leukemia virus. Dr. Hartley said her laboratory employs the procedures recommended in the NIH Guidelines for handling infectious agents. The helper virus used in procedures with feline leukemia virus is not particularly infectious for humans, although it can produce lymphomas in mice.

Dr. Friedman asked whether chromosomal transposition occurs more frequently with retroviral proviruses than with DNA viruses.

Dr. Joklik said there are no specific chromosomal sites for viral integration,

although there are sequences in the viral genome necessary for integration. Dr. Mulligan said subsequent RNA copies made from the original proviral copy can be packaged and ultimately lead to new proviral insertions in different locations in the host cell chromosome(s). Most retroviruses do not usually kill the host cell but establish a more persistent infection and usually insert from one to five provirus copies in various locations in the cell's genome.

Dr. Joklik asked whether the human genome possesses sequences similar to retroviral sequences. Dr. Anderson replied that similar sequences are dispersed throughout the human genome. It is possible that recombination between retroviral sequences and similar sequences in the human genome could occur.

Dr. Rapp asked how the retrovirus HTLV III/LAV should be treated under the NIH Guidelines. He said this virus should probably be classified as a lentivirus. Dr. Joklik said the lentiviruses are retroviridieae. These viruses do not transform cells but possess reverse transcriptase activity.

Dr. Anderson asked whether HTLV III/LAV would be considered a class 2 organism. He questioned how an amphotrophic vector constructed using HTLV III/LAV genes should be handled. He said HTLV III/LAV differs qualitatively from the retroviral vectors which are now being constructed for human gene therapy and from most other retroviruses. HTLV III/LAV is replication competent while human gene therapy vectors are replication defective. HTLV III/LAV causes a fatal disease. The current amphotropic vectors constructed for human gene therapy applications are poorly contagious. Dr. Anderson said some data exist on the effect of an amphotropically packaged MoMLV vector carrying the human ADA (adenosine deaminase) gene; there is no evidence of a cytopathic effect on cells in tissue culture.

Dr. Liberman said cells of the nervous system as well as T4 lymphocytes appear to possess receptors recognized by the HTLV III/LAV virus. He said currently it is not known if other cells possess these receptors. He added that the small pool of data existing on HTLV III/LAV suggests it is not very antigenic. The data also suggest antibodies may not protect against the disease.

Dr. Gottesman asked why HTLV III/LAV differs from other retroviruses in its toxic effects.

Dr. Mulligan said HTLV III/LAV possesses a gene, the "tat" gene, which is different from genes other retroviruses possess; the "tat" gene appears to be a post-transcriptional activator.

[Rapporteur's Note: The three HTLVs infect T-lymphocytes. Infection by HTLV-I or HTLV-II, the viruses which have been associated with certain types of leukemias and lymphomas, leads to uncontrolled cell proliferation. HTLV III/LAV kills cells it infects.

All three HTLVs produce trans-activating proteins that stimulate expression of viral genes. The proteins might also alter cellular gene expression, thus disrupting growth control in infected cells. The HTLV III/LAV trans-activating gene is in a different location and distinctly different in structure and sequence from those of HTLV I and HTLV II and has a different activity.]

Dr. Grodzicker said HTLV III/LAV possesses a trans-activator gene. The DNA containing adenoviruses can also contain a trans-activator gene but adenoviruses do not usually cause cancer in humans althought they do cause cancer in rodents.

Dr. Gottesman asked whether adenoviruses might not cause cancer in animals because they rapidly kill cells rather than transform them. Dr. Grodzicker said rapid cytotoxicity is one explanation; the route of transmission is another possible cause.

Dr. Joklik said many leukemia viruses such as Rous associated virus (Rav) II are very cytotoxic. The basis for the cytotoxicity of these retroviruses is not known although it has been shown that there is strain and envelope control of cytotoxicity.

Dr. Gottesman asked why the incidence of disease caused by HTLV I is lower than the incidence of disease for HTLV III/LAV. Dr. Rapp said HTLV I infection rarely leads to disease; since neoplasia is a multistage process, it is assumed other cellular events in addition to HTLV I infection are needed to cause disease. Infection by HTLV III/LAV may be sufficient to cause disease.

Dr. Friedman asked whether the working group should write specific language on viruses for the NIH Guidelines. Would this language apply to both DNA and RNA viruses? Would a special case have to be made for HTLV III/LAV?

Dr. Landy thought such an approach is too simplistic, and the working group would not be able to write language addressed to combinations of viral genes. Dr. Grodzicker agreed it would be difficult to develop language applicable to all cases.

Dr. Gottesman asked the working group if they thought a comprehensive listing of retroviruses in Appendix B should be developed or would descriptive language be more useful in the NIH Guidelines. She noted that oncogenic viruses are not currently classified in Appendix B although some are listed. Dr. Grodzicker thought that some viruses currently listed in Appendix B do not present a hazard and should not be in Appendix B.

Dr. Liberman suggested it might be reasonable to base a listing on host range. He said a number of organisms which pose no hazard to humans (e.g., mouse ecotropic retroviruses) would then drop out of the listing.

Dr. Liberman added that the American Type Culture Collection (ATCC) has the most extensive hazard listing of a retroviral collection because they must comply with international shipping regulations. The ATCC listing might be helpful to the working group should they decide to construct an Appendix B listing.

Dr. Gottesman said the National Cancer Institute (NCI) has been working for two years to revise their listing of oncogenic viruses; she would contact individuals involved in this effort for a report on their progress.

The working group concluded that descriptive language in the NIH Guidelines might be more flexible and thus preferrable over a revision of Appendix B.

Dr. Gottesman said she might attempt to prepare language for RAC's consideration at a subsequent RAC meeting. She said the discussion indicated most retroviruses are either: (1) defective when they carry an oncogene; or (2) nondefective but they carry no oncogene. Rous sarcoma virus is an exception, however. This retrovirus demonstrates that a functional retrovirus can carry an oncogene. She felt the discussion indicated that BL2 containment appears in general to be appropriate for work with retroviruses. However, consideration should be given to higher containment if a broad host range, infectious, amphotropically packaged retrovirus carrying an oncogene is being studied.

Dr. Anderson said Dr. Gottesman should address two separate issues in attempting to draft language; the use of retroviral vectors in human gene therapy and the use of wild-type viruses in other types of procedures.

Dr. Mulligan said in human gene therapy the investigator attempts to introduce the vector into the cells of human subjects. Two issues should be addressed for use of retroviral vectors in human gene therapy: (1) could new viruses be generated; and (2) would integration into the human genome disrupt or activate important chromosomal sequences. Dr. Gottesman said consideration should also be given to the possibility of genomic rearrangements in human gene therapy.

[Rapporteur's Note: Retroviral vectors can rearrange their own structure as well as exchange sequences and could recombine with an endogenous retroviral sequence or with other retroviruses. At present, it is thought possible that a retroviral sequence might recombine with an endogenous retroviral sequence to produce packageable, infectious recombinant virus. The properties such a recombinant would possess are unknown, but the potential homology between retroviral vectors and as yet unknown primate retroviruses or human T-cell leukemia viruses might be sufficiently close to produce recombinants. There is, however, a built-in safety feature when mouse retroviral vectors are used. These mouse virus structures have very different sequences from known primate retroviruses, and there appears to be little homology between the two. Recombination frequencies thus would be very low or negligible.

The second potential hazard posed by the use of retroviral vectors in human gene therapy is mutagenesis as a result of provirus integration into the chromosome. Because the provirus carries transcriptional regulatory sequences within the LTRs, provirus integration can result in either gene activation (insertional mutagenesis) or gene inactivation. Gene activation would be a potential hazard particularly if the provirus integrates adjacent to a cellular proto-oncogene.]

Dr. Gottesman asked the working group what is known about insertional activation or inactivation of genes by integration of provirus DNA into chromosomal sites.

Dr. Mulligan said that certain retroviruses that do not carry an oncogene can cause malignancies in animals long after their introduction into the host. How these malignancies develop from such infection is not known. It is thought in these cases a proto-oncogene is activated by provirus integration. The

process takes many months as the virus replicates through many cycles or the cells undergo further genetic changes. No evidence suggests the malignancies caused by these viruses result from a single hit event.

Dr. Gottesman asked whether leukemia would develop if viral replication did not occur. Dr. Mulligan replied that it is unlikely a proto-oncogene would be activated or inactivated without many cycles of replication. Dr. Joklik said replication is probably necessary for insertion to occur at the appropriate site in the appropriate cell. Not all cells in the animal are susceptible to tumor induction by the particular virus.

[Rapporteur's Note: The ALV provirus integrates adjacent to the c-myc gene in several B cell lymphomas and next to c-erb proto-oncogenes in leukemic erythroblast cells, resulting in elevated levels of transcription from these genes.]

Dr. LeRoy Walters of the Kennedy Institute and Chair of the RAC Working Group on Human Gene Therapy asked how many proto-oncogenes are in human cells. Dr. Mulligan said there are at least 30 proto-oncogenes in the human genome and probably more.

Dr. Anderson thought most retroviral insertions would not occur close enough to a proto-oncogene to activate it. The distance of the insertion site from a proto-oncogene and its regulatory signals is probably the primary factor affecting activation or inactivation of the proto-oncogene.

Dr. Anderson said for human gene therapy the control regions of the retroviral vector will probably be eliminated; the vector sequences thus would not be able to override the chromosomal regulatory signals near the insertion site. Dr. Mulligan noted that even if such vectors were utilized, the transcriptional control signals used to promote expression of the inserted genes might activate adjacent cellular genes as well.

Dr. Mulligan said in addition to insertion, additional events involving the deletion and/or rearrangement of viral sequences are probably necessary for gene activation.

[Rapporteur's Note: Several but not all of the ALV-induced B cell lymphomas result from activation of the c-myc gene by promotor insertion: the ALV proviruses had integrated into sequences upstream of the oncogene such that myc transcripts were initiated from the promotor within the 3' LTR. In most other tumors examined, however, provirus integration occurs either in an orientation transcriptionally opposite to that of the putative oncogene, or downstream of the gene within the 3' flanking sequences. In these cases, the provirus may be activating cellular gene expression by means of cis-acting transcriptional enhancer elements in the LTR.

It is thought deletion of the promotor and enhancer sequences within the U3 region of the 3' LTR should eliminate the ability of a retrovirus provirus, even if it did integrate next to a cellular proto-oncogene, to activate that gene.]

Dr. Anderson said bone marrow cells rendered malignant by gene therapy vectors could probably be recognized in an actual clinical protocol since the transformed cells would overgrow in tissue culture.

Dr. Gottesman asked whether any data exist on gene activation of cells in tissue culture by retroviral insertion. Dr. Mulligan said the frequency of activation of proto-oncogenes by retroviruses in tissue culture is probably approximately 10^{-7} in tissue culture systems.

Dr. Gottesman asked whether there is any evidence a human gene therapy vector might integrate at a specific site.

The working group said no current data demonstrate site specific integration of vectors in mammals. Homologous site-specific integration occurs at a very low level, when it occurs at all, in mammals. Retroviruses insert at random sites in the chromosome since retroviral integration does not depend on homology.

Dr. Gottesman asked whether deletion mutation would result with a higher frequency upon integration than would insertional activation.

Dr. Anderson said deletion mutation is not observed on tissue culture plates with 10^7 cells per plate. The frequency of deletion mutation may be below this detection limit. Several investigators have looked for deletion mutation and have not been able to demonstrate it.

Dr. Landy asked whether evidence suggests tumor formation can result from gene inactivation. Dr. Mulligan said human retinoblastoma is a genetic disease in which it is believed that the inactivation of both alleles of a gene play some role in tumor formation. Deletion of the alleles of a gene which may be an anti-oncogene has been observed in some of these tumors.

[Rapporteur's Note: Inactivation of genes by provirus integration has been observed in both somatic cells in culture as well as in germ cells in vivo. For example, in some in vitro experiments, it was shown that the v-src gene of the Rous sarcoma virus tranformed rat cell line B31 could be inactivated by insertion of a MoMLV provirus insertion. In addition, infection of a pre-implantation mouse embryo has led to insertion of a MoMULV provirus into the collagen gene. In the case of the mutated B31 cell line, reconstitution of src gene expression was associated with excision of most of the MoMLV provirus.

Dr. Gottesman asked whether gene therapy vectors could recombine with endogenous sequences to generate an infectious virus containing human cloned DNA. Could a recombination involving foreign cloned DNA, viral replication, and encapsidation functions result in a new virus?

Dr. Anderson said MoMLV is currently the vector of choice for human gene therapy. Recombination between the human genome and MoMLV is minimal even if the vector is packaged amphotropically. There is some homology between MoMLV sequences and the human genome under low stringency conditions; the only region of MoMLV somewhat homologous to human endogenous viruses is the pol region which displays 40% homology. This region is deleted from the vectors designed for use in human gene therapy. Dr. Anderson said Drs. Malcolm Martin and Steve Goff of the NIH had published a letter in Science stating they tried to recombine MoMLV and human endogenous sequences and failed. Therefore, a potentially "safe" proviral vector construct might be composed of mouse LTRs with their enhancer and promoter regions deleted and a human gene controlled by the appropriate human genomic regulatory signals.

Dr. Mulligan said the issues facing investigators constructing human gene therapy vectors is how to obtain high titers of helper-free virus, efficient infection of cells and a stable vector capable of expressing the desired gene in appropriate cells. As a consequence of retroviral properties, there is strong selection in infectious retroviruses for particular types of sequence organization and for the absence of certain sequences. In addition, the level of expression of genes with internal promotors relative to the level of virus production varies with the details of vector construction often in an unpredictable fashion.

[Rapporteur's Note: Retroviruses normally must modulate the amount of splicing of full-length viral RNA so that proper amounts of RNA are available for these functions: (1) full length retroviral RNA for use as mRNA for gag and pol genes; (2) subgenomic mRNA for env gene expression; and (3) full length RNA for packaging into progeny virions. It is not surprising that insertions and deletions would influence the amount of subgenomic RNA produced.]

Dr. Gottesman asked how HTLV III/LAV controls transcription. Dr. Mulligan said HTLV III/LAV has not only acquired additional genetic information but has also evolved a very complex splicing mechanism. He said many rules concerning the processing of retroviral transcripts are not yet understood.

Dr. Gottesman said the NIH Guidelines currently treat the association of helper virus and defective virus as infectious. Defective retroviruses can persist in association with helper virus. Should this situation be specifically addressed by the NIH Guidelines?

Dr. Mulligan said generally the defective retrovirus/helper virus situation is not stable. For example, with embryos in tissue culture the infection of defective mutant and helper virus spreads through the embryo; however, after several replication cycles, defective viruses can no longer be detected.

Dr. Joklik said one case is known in chickens in which the helper and defective virus situation is stable. All sorts of rearrangments occur but follow very strict rules as to what is permitted. At the moment, we do not know these rules.

Dr. Hartley said mouse retroviruses cause an ecotropic spreading infection with regular generation of a spectrum of recombinants between ecotropic and endogenous sequences. This activity is host organ dependent in mice occurring

almost exclusively in the thymus. It does not occur in fibroblast cells in culture. Some of these recombinations occur in the env region with resulting alterations in host range. Viruses with extended host ranges occur through recombination between endogenous sequences, which are expressed during high level replication of a spreading virus, and infecting virus. Generally the host range of the resulting recombinant virus can be predicted from knowledge of the host and the virus. If the host range of two infecting viruses are known, predictions of the host range of recombinants can be made.

Dr. Gottesman asked whether a unique virus could be generated in mice through recombination of a viral vector and an endogenous virus or viral sequence. Dr. Hartley replied the probability of obtaining a recombinant is low for one cycle of replication. The probability rises with the number of replication cycles. Such a situation would not be unique to recombinant DNA derived viruses.

Dr. Liberman asked whether it would be possible to certify vectors for human gene therapy as had been done for other host-vector systems. (See Appendix E of the NIH Guidelines.) He did not foresee a need for a great number of vectors and thought certification of the vectors most likely to be used in human gene therapy protocols might simplify and speed-up review of protocols.

Dr. Gottesman said in the past NIH has certified vectors; some problems, however, developed when better vectors than those which had been certified were subsequently developed. Each protocol for human gene therapy will have to be carefully evaluated on a case-by-case basis by the Working Group on Human Gene Therapy and the RAC. As part of this review, the retroviral vector constructs will be carefully scrutinized.

Dr. Liberman asked whether data on the vectors could be generated in advance of submission of the first human gene therapy protocol. Prior review of such data would also speed-up the review process. He suggested the Working Group on Viruses should also review the biology of some of the retroviral vectors most likely to be used in human gene therapy.

Dr. Gottesman said the Working Group on Human Gene Therapy could receive and review in vitro data on the vectors before submission of clinical protocols.

Dr. Anderson said investigators are usually developing, constructing, and testing several vectors simultaneously. Investigators do not wish to submit data on every vector; most of these vectors will not be used in actual human gene therapy protocols. Dr. Gottesman said investigators would not be required to submit such data, rather they may submit the data if they wished.

Dr. Simpson asked Dr. Walters whether RAC will provide guidance to IBCs in reviewing the retroviral vectors used in human gene therapy. He said retrovirology itself is a complex subject and still greater expertise is required to evaluate the vector constructs.

Dr. Anderson said retroviral vectors are very intricate; the type of expertise and information necessary to evaluate these vectors may not be available to IBCs and Institutional Review Boards (IRBs). He asked Dr. Walters if the

Working Group on Human Gene Therapy would form a panel of retrovirology experts to review protocols.

Dr. Walters said it is possible the Working Group on Human Gene Therapy would form such a panel of experts. Both the RAC and the working group should be educated in the science of retroviral vectors.

Dr. Simpson said the requirement that IBCs and IRBs must review the protocols before the proposal is submitted to RAC for review puts pressure on IBCs and IRBs since they cannot send a "shoddy" review to RAC. He thought IRBs and IBCs would probably wish to have more guidance.

Dr. Walters said the Working Group on Human Gene Therapy has prepared points to consider for evaluating human gene therapy protocols. These points to consider will be sent to IBCs. He hoped his working group will be able to supply more detailed information for evaluating the vector constructs and safety considerations.

Dr. Gottesman said the Working Group on Human Gene Therapy is willing to assist IRBs and IBCs in protocol review.

Dr. Anderson said another issue should be addressed by the Working Group on Human Gene Therapy; protocols must be submitted to IRBs and IBCs before submission to the RAC. This process will require a certain amount of time. If the protocols were submitted to NIH before IRB review is complete, could IRBs accept the amount of publicity associated with the NIH review process?

Dr. Gottesman adjourned the meeting of the Working Group on Viruses at 3:20 p.m. on November 12, 1985.

[Rapporteur's Note: Primary reference sources used for expanding upon certain portions of the discussion are cited in Attachment III].

Respectfully submitted,

Elizabeth A. Milewski, Ph.D.
Rapporteur

Stanley Barban, Ph.D.
Executive Secretary

I hereby certify that, to best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

1/27/86
Date

Susan Gottesman, Ph.D.
Chair

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service
National Institutes of Health

Memorandum

Date November 8, 1985

From Executive Secretary
Recombinant DNA Advisory Committee

Subject Proposed Modifications of the Guidelines to Include Reference to Recombinant RNA

To The Record

Drs. Gottesman, Martin, Talbot, and I spoke by conference call on November 4, 1985, to consider possibilities for including specific reference to recombinant RNA in the NIH Guidelines for Research Involving Recombinant DNA Molecules. This arose from discussions by the Recombinant DNA Advisory Committee as to whether there should be an explicit statement that retroviruses are covered by the Guidelines, particularly in the case of human gene therapy protocols, which require review and specific approval by the NIH under Section III-A of the Guidelines.

After discussing alternative ways of including reference to recombinant RNA in the Guidelines, it was proposed that reference to recombinant RNA be added to sections III-A-4, III-B-3, and III-B-4-a. The sections would be revised to read as follows (revised wording is underlined):

III-A-4. Deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA into human subjects (21). The requirement for RAC review should not be considered to preempt any other required review of experiments with human subjects. Institutional Review Board (IRB) review of the proposal should be completed before submission to NIH.

III-B-3. Experiments Involving the Use of Infectious Animal or Plant DNA or RNA Viruses or Defective Animal or Plant DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems.

Caution: Special care should be used in the evaluation of containment levels for experiments which are likely to either enhance the pathogenicity (e.g., insertion of a host oncogene) or to extend the host range (e.g., introduction of novel control elements) of viral vectors under conditions which permit a productive infection. In such cases, serious consideration should be given to raising the physical containment by at least one level.

Note.--Recombinant DNA molecules or RNA molecules derived therefrom which contain less than two-thirds of the genome of any eukaryotic virus (all virus from a single Family (17) being considered identical (19) may be considered defective and can be used in the absence of helper under the conditions specified in Section III-C.

III-B-4-a. Recombinant DNA or RNA molecules derived therefrom from any source except for greater than two-thirds of a eukaryotic viral genome may be transferred to any non-human vertebrate organism and propagated under conditions of physical containment comparable to BL1 and appropriate to the organism under study (2). It is important that the investigator demonstrate that the fraction of the viral genome being utilized does not lead to productive infection. A USDA permit is required for work with Class 5 agents. (18, 20).

It is proposed that the RAC consider these proposed changes at its meeting on January 27, 1986.

William J. Gartland, Jr., Ph.D.

RAPPORTEUR'S PRIMARY REFERENCE SOURCES

The rapporteur's notes were based primarily on the following articles:

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National Institutes of Health

Recombinant DNA Research; Action Under Guidelines

AGENCY: National Institutes of Health (NIH), PHS, DHHS.

ACTION: Notice of action under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth an action taken by the Director, NIH, under the November 1984 NIH Guidelines for Research Involving Recombinant DNA Molecules (49 FR 46266).

EFFECTIVE DATE: November 13, 1985.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20892 (301) 496–6051.

SUPPLEMENTARY INFORMATION: Today an action is being promulgated under the NIH Guidelines for Research Involving Recombinant DNA Molecules. In accordance with Section IV-C-1-b of the NIH Guidelines, this action has been found to comply with the NIH Guidelines and to present no significant risk to health or the environment.

I. Approval to Field-Test Disease Resistant Tobacco Plants

In a submission dated May 5, 1983, Dr. Winston Brill of Cetus Madison Corporation (CMC) requested approval under Appendix L of the NIH Guidelines for Research Involving Recombinant DNA Molecules to field test plants grown from seeds containing recombinant DNA. CMC and its successor, Agracetus, do not receive any support for recombinant DNA research from the NIH and, therefore, are not required to comply with the NIH Guidelines. All submissions by CMC were made voluntarily under Part VI of the NIH Guidelines. A review by the Plant Working Group of the NIH Recombinant DNA Advisory Committee (RAC) concluded that the submission did not meet the criteria required for approval under Appendix L of the NIH Guidelines and would, therefore, require review not only by the RAC Plant Working Group but also by the full RAC.

On July 1, 1983, CMC submitted a revised proposal to field test plants grown from seeds containing recombinant DNA. After review by the Plant Working Group, additional information was requested.

On August 19, 1983, CMC submitted a revised proposal to field test plants engineered by recombinant DNA techniques. The proposal was reviewed by the RAC Plant Working Group on September 7, 1983, and by the full RAC on Septebmer 19, 1983. At its September 19, 1983 meeting, the RAC passed a motion by a vote of 12 in favor and 1 opposed. The motion stated that while the proposal was acceptable in principle, it required further amplification and clarification before approval.

On October 21, 1983, Dr. Brill provided further data on the field design and the types of observations to be made and also limited the request to tobacco plants.

On December 21, 1983, the RAC Plant Working Group unanimously recommended approval of the request as described in the submissions from CMC of August 19, 1983, and October 21, 1983.

On January 24, 1984, the U.S. Department of Agriculture (USDA) Recombinant DNA Committee unanimously concurred with the recommendation of the NIH RAC Plant Working Group.

The proposal was reviewed by the RAC at its June 1, 1934, meeting. The RAC with no negative votes recommended approval of the proposal.

On August 30, 1984, NIH was informed that the business of CMC had been transferred to a partnership having the name "Agracetus."

On September 14, 1983, a lawsuit was filed (Foundation on Economic Trends, et al. v. Margaret Heckler et al., Civil Action #83–2714 in the United States District Court for the District of Columbia). On May 16, 1984, Judge John Sirica issued a preliminary injunction in this case. The injunction was partly affirmed and partly vacated by the Appeals Court in a decision of February 27, 1985. Although the preliminary injunction specifically does not apply to proposals voluntarily submitted to the NIH (as is the case with the Agracetus proposal), I requested that an Environmental Assessment be prepared prior to my approval of this proposal. I signed an Environmental assessment and Finding of No Significant Impact on August 14, 1985.

Both the material submitted by Agracetus and the Environmental Assessment contain material identified by Agracetus as proprietary information.

I accept the recommendation of the RAC and the USDA Recombinant DNA Committee; and Appendix D of the NIH Guidelines is amended to reflect this decision.

II. Summary of Action

The following new section is added to Appendix D of the NIH Guidelines:

Appendix D–XI. Agracetus of Middelton, Wisconsin, may field test under specified conditions disease resistant tobacco plants prepared by recombinant DNA techniques.

Dated: September 25, 1985.

James B. Wyngaarden, M.D.,

Director, National Institutes of Health.

[FR Doc. 85–26916 Filed 11–12–85; 8:45 am]

BILLING CODE 4140–01–M

ENVIRONMENTAL ASSESSMENT
AND
FINDING OF NO SIGNIFICANT IMPACT

Concerning the application to the National Institutes of Health (NIH) under the NIH Guidelines for Research Involving Recombinant DNA Molecules by Agracetus of Middletown, Wisconsin, to field test disease resistant tobacco prepared by recombinant DNA techniques.

National Institutes of Health
Public Health Service
U.S. Department of Health and Human Services

Concurrence: James B. Wyngaarden

James B. Wyngaarden, M.D.
Director
National Institutes of Health
Date: 8-14-85

NOTE: This EA-FONSI differs from the one signed on August 14, 1985, by the deletion of material which would describe the exact location of the proposed site. Figure 3 has been eliminated, and text has been deleted on pages 19, 20, 22, 23 and Figure 2. Where such text has been deleted on pages 19, 20, 22, and 23, whether it be a word or a phrase of any length, a line of 4 spaces length has been inserted in its place.

CONTENTS

1.0 Introduction and Summary 1

2.0 Background 3

3.0 Purpose and Need 12
- 3.1 Purpose of the Proposed Action 12
- 3.2 Need for the Proposed Action 12

4.0 Description of Proposed Action and Alternatives 13
- 4.1 Proposed Action 13
  - 4.1.1 Planting 15
  - 4.1.2 Field Layout 17
  - 4.1.3 Field Observations 17
- 4.2 Alternatives to the Proposed Action 18
  - 4.2.1 Approval of Original Proposal of May 5, 1983 18
  - 4.2.2 Approval of Proposal of August 19, 1983 18
  - 4.2.3 Withhold Approval 18

5.0 Environmental Effects 19
- 5.1 The Physical Environment 19
- 5.2 Tobacco and Growing of Tobacco in Wisconsin 23
- 5.3 Crown Gall 27
  - 5.3.1 Biochemical Genetics of T-DNA 28
  - 5.3.2 Reproduction of the Plants 31
  - 5.3.3 Verification of Disease Resistance 32
- 5.4 Possible Effects of the Proposed Field Test 33
  - 5.4.1. Accidental Escape of Genetically Engineered Plants from the Test Plot 33
  - 5.4.2. Escape of Pollen or Seed of the Genetically Engineered Plants from the Test Plot 33
  - 5.4.3. Excision of the Inserted Recombinant DNA from the Genetically Engineered Plants 34
  - 5.4.4 Alterations in Palatability to Insect Pests 36
  - 5.4.5 Alterations in Palatability to Nematodes 37
  - 5.4.6 Alterations in Susceptibility to Fungal Diseases 37
  - 5.4.7 Alterations in Susceptibility to Bacterial Diseases 39
  - 5.4.8 Alterations in Susceptibility to Virus Attack 40
  - 5.4.9 Alterations in "Weediness" (Persistence of Tobacco) 41
  - 5.4.10 Pilfering from the Experimental Plot 42
- 5.5 Effects of Alternatives 43
  - 5.5.1 Approval of Proposal of May 5, 1983 43
  - 5.5.2 Approval of Proposal of August 19, 1983 44
  - 5.5.3 Withhold Approval 44
- 5.6 Summary and Conclusions 45

6.0 Consultation 46

7.0 References 49

1.0 INTRODUCTION AND SUMMARY

This document is an Environmental Assessment (EA) and Finding of No Significant Impact (FONSI) concerning the application, under the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines), by Agracetus of Middleton, Wisconsin to field test crown gall resistant tobacco prepared by recombinant DNA techniques.

This Environmental Assessment and Finding of No Significant Impact has been prepared in accordance with: (1) the National Environmental Policy Act of 1969, as amended; (2) 40 CFR Section 1501.3(b) and Section 1508.9(b) of the Council on Environmental Quality Regulations for Implementing the Procedural Provisions of the National Environmental Policy Act; and (3) Part 30, Environmental Protection, of the General Administration Manual of the Department of Health and Human Services. Authority to approve such an EA and FONSI was delegated to the Director, NIH, by the Director, Office of Management, United States Public Health Service, in his memorandum to PHS Agency Heads, dated June 2, 1980, on the subject, "Delegation of Authority for Carrying Out Responsibilities Under Revised Part 30 of the General Administration Manual, Environmental Protection."

The original submissions to NIH concerning this field test were made by Dr. Winston Brill, Director of Research, Cetus Madison Corporation, Middleton, Wisconsin. On August 30, 1984, Dr. Brill wrote to Dr. William Gartland of NIH that: "The purpose of this letter is to notify you that as of August 17, 1984 the business of Cetus Madison Corporation (CMC) was transferred to a partnership having the name 'Agracetus' between a subsidiary of W.R. Grace & Co. and CMC.

Consequently, the application by CMC... to field test recombinant DNA plants should be transferred to Agracetus, and all information previously submitted by CMC in support of its attempt to get approval for such field testing should be used to obtain approval for Agracetus."

Agracetus has used a modified T-DNA from Agrobacterium tumefaciens to induce, in tobacco, resistance to crown gall disease. They have examined, in greenhouses and growth chambers, over one thousand genetically engineered tobacco plants spanning five generations and did not observe any changes that would indicate that the proposed field test poses any significant risk to health or the environment.

When the T-DNA of A. tumefaciens is made avirulent by insertional mutation of the T-DNA cytokinin gene, tobacco plants containing this modified T-DNA are significantly more resistant to crown gall disease than those plants not genetically engineered. The genetically engineered plants are no different in growth rate, seed yield, growth habits, etc., than control tobacco plants without the modified T-DNA. So far, these tests have all been performed in greenhouses and growth chambers. There are, however, many examples of plant phenotypes expressed under field conditions that are not expressed in greenhouses. Field conditions have stresses that are not encountered in greenhouses; for example, water scarcity, water excess, temperature fluctuations, etc. For these reasons, Agracetus would like to field test these genetically engineered tobacco plants to be sure that incorporation of the modified T-DNA into plants does not debilitate them in some unexpected way.

Since T-DNA is the only vector that has been reproducibly shown to be useful for plant genetic engineering to date, field testing experience with plants modified by these vectors will help scientists gain confidence in the basic and applied research potential of plant genetic engineering and in the safety of such work. The long-term objective of all of this work is to produce crop plants with properties desirable to farmers and consumers.

Of the various plant vector constructions in use at Agracetus, they propose initially to field test Havana 425 tobacco containing T-DNA with a yeast alcohol dehydrogenase gene and a bacterial aminoglycoside phosphotransferase gene inserted in the cytokinin gene since this is the system with which they have had most experience.

The NIH has assessed the environmental effect of the proposed field test (see below in this Environmental Assessment) and has found that it will not have a significant impact on health or the environment.

2.0 BACKGROUND

The NIH Guidelines for Research Involving Recombinant DNA Molecules (Guidelines) were first issued on June 23, 1976, and were published in the Federal Register on July 7, 1976. The Guidelines were the product of a process of scientific and public discussions initiated in 1973 by scientists with concerns about the safety of the new recombinant DNA techniques. As stated in the "Decision of the Director, National Institutes of Health to Release Guidelines for Research on Recombinant DNA Molecules":

"The object of these guidelines is to ensure that experimental DNA recombination will have no ill effects on those engaged in the work, on the general public, or on the environment. . . . It should be noted that the development of the guidelines was in large part tantamount to conducting an environmental impact assessment. For example, the objectives of recombinant DNA research and alternate approaches to reach these objectives, have been considered. The potential hazards and risks have been analyzed. Alternative approaches have been thoroughly considered to maximize safety and minimize potential risk. And an elaborate review structure has been created to achieve these safety objectives."

The NIH published a Draft Environmental Impact Statement on the Guidelines in the Federal Register for public comment on September 9, 1976. After analysis of the comments received a final Environmental Impact Statement (EIS) on NIH Guidelines for Research Involving Recombinant DNA Molecules was published in October 1977.

On July 28, 1978, NIH published for public comment in the Federal Register (totalling 136 pages) proposed revised Guidelines together with "Decision of the Director, NIH, to Issue Revised Guidelines for Recombinant DNA Research," and an Environmental Impact Assessment Following analysis of the public comments received, NIH published in the Federal Register on December 22, 1978, revised Guidelines for Recombinant DNA Research," and an Enviromental Impact Assessment. The Guidelines have been revised many times since December 1978. Copies of all revisions are available from the NIH Office of Recombinant DNA Activities, Building 31, Room 3B10, NIH, Bethesda, Maryland 20205. The most recent complete republication of the Guidelines was in the Federal Register of November 23, 1984.

The NIH Recombinant DNA Advisory Committee (RAC) consists of 25 voting members, appointed by the Secretary of the Department of Health and Human Services, including laymen and scientists of many different disciplines. The RAC also has non-voting liaison representatives from the following other Federal agencies:

Department of Agriculture

Department of Commerce

Department of Defense

Department of Health and Human Services

--Office of the Assistant Secretary for Health

--National Institutes of Health

--Centers for Disease Control

—National Institute for Occupational Safety and Health

--Food and Drug Administration

--Alcohol, Drug Abuse & Mental Health Administration

--Health Resources and Services Administration

Department of Energy

Department of Interior

Department of Justice

Department of Labor

Department of State

Department of Transportation

Environmental Protection Agency

--Office of Research and Development

--Office of Pesticides and Toxic Substances

Executive Office of the President

--Office of Science and Technology Policy

--Council on Environmental Quality

National Aeronautics and Space Administration

National Science Foundation

Nuclear Regulatory Commission

U.S. Arms Control and Disarmament Agency

Veterans Administration

A list of all current and past RAC members is available from the NIH Office of Recombinant DNA Activities at the address given above.

The NIH Guidelines for Research Involving Recombinant DNA Research are primarily safety standards for laboratory research, designed to keep organisms containing recombinant DNA within the laboratory. There is a section of the Guidelines, however, which deals with "deliberate release into the environment of any organism containing recombinant DNA." (This was Section III-A, "Experiments that are not to be performed" in the original Guidelines of July 7, 1976; it was Section I-D-4 in the Guidelines of December 22, 1978; and it is Section III-A-2 in the Guidelines of November 23, 1984.)

The procedures for approval of "deliberate release into the environment" of any organism containing recombinant DNA (except for certain plants described in Appendix L of the Guidelines), are that experiments in this category cannot be initiated without submission of relevant information on the proposed experiment to NIH, the publication of information about the proposal in the Federal Register for at least 30 days of comment, review by the RAC, and a specific approval by the Director, NIH. The standard for approval of such experiments has remained the same since the December 1978 revision of the Guidelines; it is that the NIH Director "shall weigh each proposed action through appropriate analysis and consultation to determine that it complies with the Guidelines and presents no significant risk to health or the environment."

Most experiments involving deliberate release into the environment of an organism containing recombinant DNA require review by the full RAC and final approval by the Director, NIH; however Appendix L of the Guidelines specifies that field tests of plants meeting certain criteria can be reviewed by the RAC Plant Working Group rather than the full RAC, and can be approved by the NIH Office of Recombinant DNA Activities rather than the Director, NIH.

Section I-C of the NIH Guidelines states that:

> The guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an Institution that receives any support for recombinant DNA research from the National Institutes of Health (NIH).

Agracetus does not receive any support for recombinant DNA research from the NIH and therefore is not required to comply with the Guidelines. Section VI of the Guidelines, entitled "Voluntary Compliance" however encourages institutions "not otherwise covered by the Guidelines" to voluntarily comply. All submissions by Cetus Madison Corporation and Agracetus to the NIH which are discussed in this EA-FONSI were made voluntarily under Section VI of the Guidelines. Within Section VI of the Guidelines is a Section VI-E, entitled "Protection of Proprietary Data." Agracetus has indicated that material submitted to NIH contains "sensitive business information related to proprietary technologies."

The original submission of May 5, 1983, by Cetus Madison Corporation (CMC) to the NIH, requested approval under Appendix L of the Guidelines to field test cotton, soybean, corn, tobacco, tomato and potato, grown from seeds containing recombinant DNA. Following the RAC Plant Working Group's review of the original submission and additional information supplied by CMC, a letter of July 1, 1983, from Dr. William Gartland, Director, NIH Office of Recombinant DNA Activities, to Dr. Winston Brill, Director of Research, CMC, indicated the decision that the submission did not meet the criteria required for approval under Appendix L of the Guidelines, and would therefore require review not only by the RAC Plant Working Group but also by the full RAC.

On July 1, 1983, Dr. Brill submitted a revised proposal to field test cotton, tobacco and tomato, grown from seeds containing recombinant DNA. After review by the RAC Plant Working Group, a letter of July 26, 1983, from Dr. Gartland to Dr. Brill requested additional information.

On August 19, 1983, Dr. Brill submitted a revised proposal "to field test tobacco, cotton, and tomato that have been genetically engineered for resistance to the disease, crown gall." The proposal was reviewed by the RAC Plant Working Group on September 7, 1983 and by the full RAC on September 19, 1983. At its September 19, 1983 meeting, the RAC passed the following motion by a vote of 12 in favor, 1 opposed:

"The proposal by Cetus Madison to field test genetically engineered tobacco, cotton, and tomato plants, as described in their August 19, 1983, submission, was considered by RAC and, while acceptable in principle, requires further amplification and clarification before approval. RAC will empower the Plant Working Group to review and approve this proposal after further consideration of specific planting and monitoring procedures for the engineered tobacco plants and the results of greenhouse tests for engineered cotton and tomato plants."

On October 21, 1983, Dr. Brill wrote giving "further data on the field design and the types of observations to be made," and also limiting the request to tobacco.

On December 21, 1983, the RAC Plant Working Group unanimously recommended approval of the request as described in the submissions from Cetus Madison Corporation of August 19, 1983, and October 21, 1983.

On January 24, 1984, the U.S. Department of Agriculture Recombinant DNA Committee (ARAC) unanimously concurred with the recommendation of the NIH RAC Plant Working Group.

On March 15, 1984, Dr. Gartland wrote to Dr. Brill indicating an NIH decision that the proposal should be reviewed once more by the full RAC.

On April 13, 1984, Dr. Brill resubmitted the request consisting of the same material previously submitted on August 19, 1983, and October 21, 1983.

The proposal was reviewed by the RAC at its June 1, 1984, meeting. The RAC with no negative votes recommended approval of the proposal.

On September 14, 1983, a lawsuit was filed (Foundation on Economic Trends, et al. v. Margaret M. Heckler et al., Civil Action #83-2714 in the United States District Court for the District of Columbia). On May 16, 1984, Judge John Sirica issued a preliminary injunction in this case. The injunction was partly affirmed and partly vacated by the Appeals Court in a decision of February 27, 1985. Although the preliminary injunction specifically does not apply to proposals voluntarily submitted to the NIH (as is the case with the Agracetus proposal), the Director, NIH, decided that before he would approve the Agracetus proposal an Environmental Assessment should be prepared.

On April 29, 1985, Mr. Allen J. Dines, Director, Business Development, Agracetus, forwarded to the NIH both additional details concerning the proposed field test and a draft environmental assessment. That draft EA was revised by the NIH to produce this EA.

On July 8, 1985, Mr. Allen J. Dines sent a letter to the NIH indicating that the proposal already recommended for approval by the RAC had also been submitted for review to the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture. Mr. Dines included with his letter a copy of a June 14, 1985, letter to him from James W. Glosser, Acting Administrator, APHIS, which states that: "Based upon our review of your proposal, we have determined that your genetically engineered tobacco plant does not present a plant pest risk. Therefore, your proposed field testing would not be subject to further regulation by USDA, APHIS, under the Plant Pest Act."

3.0 PURPOSE AND NEED

3.1 Purpose of the Proposed Action

The purpose of the proposed field test is to determine whether the alterations in the genetic material of the experimental tobacco have any detrimental effect on growth or characteristics of the tobacco.

The purpose of NIH approval of this proposed field test is, as set forth in the Guidelines, to determine that the test complies with the Guidelines and presents no significant risk to health or the environment.

3.2 Need for the Proposed Action

Growth of the experimental tobacco has been compared with the parent tobacco in the confinement of a greenhouse under controlled conditions. Such tests, however, cannot duplicate the rigors of field testing and exposure to the variety of stresses present in the natural environment. If genetic engineering of plants is to provide material of value to agriculture, the engineered plants, like conventionally produced genetic alterations, must be tested under field conditions.

The NIH review and approval of this proposed field test is being conducted under the "Voluntary Compliance" section of the NIH Guidelines to provide review at the national level of whether the test can be conducted safely with no significant risk to health or the environment.

4.0 DESCRIPTION OF PROPOSED ACTION AND ALTERNATIVES

4.1 Proposed Action

The proposed action is NIH approval of the field test proposed by Agracetus in their submission of August 19, 1983, with additional information supplied on October 21, 1983, and April 29, 1985.

This will involve field testing of Havana 425 tobacco containing T-DNA with a yeast alcohol dehydrogenase gene and a bacterial aminoglycoside phosphotransferase gene inserted in the cytokinin gene. In the proposed field test the genetically engineered tobacco will be grown at the test site in Wisconsin in alternate rows with the unaltered parent, Havana 425, in a plot of about 0.02 hectares (0.05 acres) surrounded by soybeans (Figure 1). After harvest, plant material will be removed from the plot and will be destroyed by autoclaving. Roots and stumps of field tobacco are normally killed by freezing and do not survive a Wisconsin winter. Such material can be removed from the plot and autoclaved if for any reason such action seems warranted. Seed production by the tobacco in the plot will be prevented by removing tops of the plants ("topping," the normal practice for Wisconsin tobacco). Seed will be saved from a small number of experimental plants and controls. The flowering stalks of such plants will be enclosed in cloth bags before flowers open to prevent entry or escape of pollen.

The plants to be tested are tobacco grown from seeds containing T-DNA modified by genetic engineering to "destroy" the cytokinin gene.

The fields will have controlled access and good cultural and pest control practices will be used. The crown gall resistant tobacco will be isolated from plants of the same species outside of the experimental plot in accordance with pollination characteristics (Federal Seed Act; 7 CFR, Section 201.76).

Agracetus' Institutional Biosafety Committee reviewed this proposal which included appraisals by scientists and consultants knowledgeable of the crops, their production practices, and the local geographic conditions where they will be grown.

Any observation that might indicate that a crown gall resistant tobacco has a detrimental effect on other plants, animals, humans or the environment will be reported immediately to the Director of Research of Agracetus. Any problems caused by genetically engineered plants will be reported promptly to the Director of the NIH Office of Recombinant DNA Activities and the Chairman of the USDA Recombinant DNA Committee. Members of the Agracetus IBC and managers of neighboring fields also will be notified.

If there is any indication that something unusual is being transmitted specifically from the genetically engineered crown gall resistant plants to neighboring plants, the plants will be dug up and destroyed by autoclaving or burning. In that event, the plants in the surrounding area would be tested the next growing season to determine if those plants had acquired the foreign DNA. The soil in which the genetically engineered plants were grown can be fumigated if required.

It is important to stress, in this regard, that the crown gall resistant genetically engineered plants will differ from the non engineered plants only by the addition of a very small amount of well-characterized foreign DNA.

Neither the seed nor any other part of the plant will be fed to humans or farm animals.

4.1.1 Planting

Plants for field testing of crown gall resistant tobacco will be raised in Agracetus' greenhouses until about the 4-leaf stage. Plants in flats will then be moved out-of-doors adjacent to the greenhouses to "harden" them before transplanting. Transplanting is the usual commercial practice for tobacco. All necessary practices for obtaining disease-free plants for field testing will be followed. The field for this trial will be leased private land near Agracetus and under their control. The nearest commercial tobacco field will be at least 10 miles from the experimental plot.

Field testing will include the parental cultivar (Havana 425) and the genetically engineered crown gall resistant line. All plants will be observed and recorded in the plot at least once a week during the growing season. To provide critical comparison of the two treatments, only single row plots will be used. Details are given below:

1.	Number of Treatments	=	2
2.	Field Design	=	Randomized complete block
3.	Replicates	=	12
4.	Plot Size: Row Length	=	23'
	Rows/Plot	=	1
5.	Distance Between Rows	=	38"
6.	Distance Between Plants (within a row)	=	18"
7.	Plants/Row (= Plot)	=	12 (plus 2 parental types on each end of each row)
8.	Fertilizer/Lime	=	According to soil test
9.	Weed Control	=	Pre-transplant herbicide and mechanical weeding will be used (to keep the test completely free from weeds)

4.1.2 Field Layout

Figure 1 details the layout of the field plants for this proposed test. The test will have border rows of parental-type tobacco. After providing a 10' alley, the test will be surrounded on all sides by two rows of parental-type tobacco. In addition, two plants of parental type will be planted on both ends of each row. To virtually eliminate pollen dispersal, all but five of the plants will be harvested prior to flowering. Five plants will be kept in the field until maturity for seed collection. Field test personnel will bag the flowers of these five plants to prevent dispersal of their pollen. The plants will be 3' apart up to 100', and the spacing will increase to 5' up to 200', and 10' thereafter. Both lines flower synchronously in greenhouse trials.

4.1.3 Field Observations

The test will be carefully monitored at all stages (from 4-leaf through maturity) of plant growth of the crown gall resistant and crown gall sensitive tobacco. Agracetus agronomists will visit the field at least once a week. Agracetus molecular biologists, plant pathologists, plant physiologists, and consultants in plant pathology, horticulture, and entomology will also monitor the field trial. Other University of Wisconsin experts may visit the test site, if needed.

At harvest, data on the crown gall resistant and sensitive tobacco will be recorded on leaf yield, plant height, texture and thickness of leaves, and other traits. Quantitative data will be subjected to analysis of variance, and the statistical significance of the character means will be tested.

More detail on field test protocols is provided in the Field Testing Plan submitted to NIH on April 29, 1985.

4.2 Alternatives to the Proposed Action

4.2.1 Approval of Original Proposal of May 5, 1983

Under this alternative the NIH Director would approve field tests on cotton, soybean, corn, tomato and potato in addition to tobacco.

4.2.2 Approval of Proposal of August 19, 1983

Under this alternative, the NIH Director would approve field tests on cotton and tomato in addition to tobacco.

4.2.3 Withhold Approval

Under this alternative the NIH Director would not approve the proposal of Agracetus recommended for approval by the NIH Recombinant DNA Advisory Committee at its June 1, 1984, meeting.

5.0 ENVIRONMENTAL EFFECTS

5.1 The Physical Environment

The proposed field test site is located within ___ of Agracetus' facilities in ___, Wisconsin. The test site is approximately 0.05 acres and is buffered by 10 acres of surrounding soybeans (Figure 2). This test site represents half of the acreage at the Agracetus experimental farm site. The other 10 acres, which form the southern boundary for the test site, will be planted in Agracetus experimental hybrid corn. The whole 20 acre site will be surrounded by hybrid corn from neighboring farmers' fields (Figure 2).

The test site is located approximately ___ (Figure 3). This area is largely agricultural, with the rural setting interrupted only by a ___ and a scattering of other ___ buildings and rural homes. The soil in the area is predominantly of the Batavia-Houghton-Dresden Association (27). These soils are rich silt loams and mucks, underlain by silt, sand and gravel. Surface soils in the area are slightly acid, ranging from pH 5.8 - 6.5.

The test site is located in the drainage basin of ___, which flows into ___.

Agricultural Use

Out of ___ total acres in the Township of ___ acres are used as farmland (___ percent). Two-thirds of the agricultural acreage is planted in three major crops, corn, alfalfa hay, and oats. The data provided in the following table summarizes the land uses in the Township of ___ (29).

TOWNSHIP OF ___ LAND USES

AGRICULTURAL CROPS	ACRES	
Field Corn	___	
Alfalfa Hay	___	
Oats	___	
Other Crops	___	
Total Agricultural Acreage		___
NONAGRICULTURAL LAND USE		
Residential	___	
Commercial-Retail	___	
Industrial	___	
Undeveloped	___	
Other Nonagricultural Uses	___	
Total Nonagricultural Land Use		___
Total ___ Acreage		___

Pesticide Usage

Since corn and alfalfa hay are the predominant crops in the area, pesticide usage concentrates on pests which afflict these two crops. The major insect problems are the corn rootworm and the corn borer. Farmers primarily use Counter, Dyfonate, Furadan, Lannate, Diazinon and Sevin to control these pests. For weed control, Atrazine, Bladex, Dual, Simazine Lasso and Metribuzin are the major pesticides used.

Climatic Conditions

The table below presents 1984 average climatic data for May-October, the months in which the field test will take place (21).

Month	Average Maximum Temp (Degrees F)	Average Minimum Temp (Degrees F)	Days of Frost	Sunshine (Percent)	Average Wind Speed (MPH)
May 1984	65.4	41.3	3	49	9.5
June 1984	78.5	56.5	0	64	9.7
July 1984	81.5	58.8	0	66	7.9
August 1984	83.3	59.3	0	71	7.4
September 1984	71.5	47.0	4	60	9.5
October 1984	62.1	41.8	8	38	10.1

Frost days are those where the overnight recorded low is less than 32°F. In a normal year, the last spring frost is around May 15. In the fall, the first frost is usually toward the end of September. Summertime maximum temperatures are in the low eighties, often associated with a high relative humidity.

Surrounding Animal and Human Populations

The Township of ___ is a largely undeveloped area with a population of ___. Only ___ percent of the area in the Township is in residential development. Over ___ percent of the acreage is either undeveloped or used agriculturally (8). The test site is approximately ___ from the western edge of ___.

Since the Township of ___ is predominantly agricultural, there are no major forested areas in the vicinity. Only ___ percent of the Township of ___ acreage is still in woodlands (8). Tree species commonly found in this area are: six species of native oaks-black, bur, white, swamp white, hill's and red; paper and river birch; shagbark hickory; boxelder; hawthorn; willow; tamarack; silver maple; basswood; black cherry; American elm; and trembling aspen (23).

Wildlife in the area represents a cross-section of typical southern Wisconsin animal life. Because of the lack of heavily forested areas, there is a scarcity of large animals. Common species include: opossum; striped skunk; wood chucks; ground, large-tree and flying squirrels; muskrat; gray and red fox; raccoon; badger; cotton tail rabbit and white-tail deer (13).

Birdlife in the Township of ___ is varied and extensive due to the diverse terrain consisting of woodlands, pastures, farmers' fields, rivers, lakes, and swamps. Species common during the year include: Canadian goose; loon; blue heron; common egret; mallard, black, wood and ring-necked ducks; marsh, red-tailed and sparrow hawks; ruffed grouse; ring-tailed pheasant; bobwhite; mourning dove; barn, barred, and great horned owls; whippoorwill; hairy, downy, and red-headed woodpeckers; tree and barn swallows; and the purple martin (11).

The nearest large concentration of fish is in ___ of the test site. Major species in this lake are perch, crappies and walleye pike.

5.2 Tobacco and Growing of Tobacco in Wisconsin

Tobacco (Nicotiana tabacum) is a plant which is native to the Western Hemisphere from Mexico southward. The Indians of the Yucatan were cultivating tobacco when the Spaniards first arrived in 1519. Europeans began growing tobacco in Spain and France by 1561. American tobacco culture began in 1612 in Jamestown, Virginia. Tobacco is now grown in a wide range of climatic and soil conditions in the United States (19).

Tobacco belongs to the Solanaceae family, commonly called the nightshade family. This family consists of over 75 genera and 2,000 species. Members of this family are predominantly tropical plants, but they are also well represented in temperate regions (2).

Within the Solanaceae family, tobacco belongs to the Nicotiana genus. This genus consists of over 70 species, mostly ornamentals such as alata, glauca, sylvestus and sandrae (1). Only two species of Nicotiana are cultivated for their leaves, tobacco (N. tabacum) and Indian tobacco (N. rustica). Indian tobacco is native to the United States but is predominantly grown in foreign countries because of its high nicotine content. Consequently, Indian tobacco has little economic impact on this country.

Tobacco, however, has a significant economic impact in the United States as this country leads the world in tobacco production. Farmers in the United States plant over 860,000 acres in tobacco with the farm value of this crop exceeding 1.4 billion dollars (19).

N. tabacum is believed to originate from a natural cross between N. sylvestrus and another native species. The plant is four to six feet high, terminating in a cluster of over 150 funnel-shaped flowers. The tobacco fruit is a capsule that splits open at maturity, containing up to 8,000 seeds. The tobacco plant bears 12 to 15 leaves that are up to two feet long (19).

There are several thousand varieties of tobacco. Havana 425, the variety to be field tested, was introduced in 1955 by a University of Wisconsin plant pathologist because of its resistance to the tobacco mosaic virus and black root rot. However, Havana 425 is seldom grown today. Havana 425 was replaced by other varieties which had similar or better yields, improved disease resistance and lacked this variety's susceptibility to wind damage.

Agracetus chose Havana 425 for laboratory research because it is well suited to recombinant experimental use and one can easily obtain callus growths.

Tobacco has been grown in Wisconsin for well over a hundred years with no known adverse ecological effects. The size of the crop varies from year to year depending on the market price and allotment quotas. Experimental tobacco is exempt from allotment restrictions as long as it is not marketed. In 1983, Dane County, Wisconsin harvested 3,080 acres of tobacco, mostly in the southern and eastern parts of the county. The proposed plot is an insignificant addition to the total tobacco grown.

Most of the tobacco grown in Wisconsin at this time contains genetic fragments of other Nicotiana species, introduced by more conventional means to provide resistance to diseases. For example, a small chromosome segment, essentially a single gene, transferred from N. glutinosa (12) conditions a hypersensitive response to tobacco mosaic virus. This response localizes the virus near the point of infection and effectively eliminates spread of the virus within the plant and between plants. Over 90 percent of

the tobacco crop consists of varieties with this sort of resistance. Havana 425 carries this resistance, and it is present in the experimental tobacco.

Additionally, a small segment of a chromosome from N. longiflora confers resistance, in certain varieties, to race O of Pseudomonas syringae pv. tabaci (7). Over 90 percent of the commercial tobacco in Wisconsin has this type of resistance, in addition to resistance to tobacco mosaic virus, but Havana 425 and the genetically engineered tobacco to be tested in this proposed field test do not have this resistance. As far as is known, these additions to the genetic complement of tobacco had an effect only in regard to the response of the crop to these diseases. There is no reason to suppose that resistance to Agrobacterium tumefaciens in the experimental tobacco to be tested in this proposed field test would have any effect other than resistance to Agrobacterium tumefaciens.

The crown gall disease caused by A. tumefaciens is not known to occur in field tobacco. It is highly unlikely that the genetic alterations would affect the interactions of the experimental tobacco with other microorganisms or viruses.

5.3 Crown Gall

Agrobacterium tumefaciens is a bacterium that causes a disease known as crown gall in many species of dicotyledonous plants. The disease is characterized by production of nondifferentiated growth that is not self-limited. Crown gall disease causes crop losses, most marked in horticultural stone fruits (e.g. plums, peaches) and in certain ornamental crops. Galls may occur on the crowns, leaves, and stems. The galls may range from a millimeter to about 20 centimeters in diameter and from less than one ounce up to 100 pounds in weight. Losses occur primarily at tree nurseries due to injury caused by grafting, pruning, and harvesting. Infected plants can become stunted and subject to blow over during windstorms.

Wounds are required for penetration of the host plant by the pathogen and for altering the host cells to a susceptible state. Wounds may occur by insects and from cultural practices such as root pruning of nursery stock. A. tumefaciens, introduced into tissues by vacuum infiltration in the absence of wounding, does not produce galls. Host susceptibility is dependent on the type of the plant, age of the plant, and the site of infection. Younger tissues generally are more susceptible than older tissues.

One hundred thousand to one million cells of the A. tumefaciens pathogen are required to cause a gall in a single plant. A. tumefaciens has been isolated from pasture lands that had never been tilled or cultivated; therefore, the organism is expected to be found in most soils. Soils with

crown gall diseased plants have increased populations of A. tumefaciens, with galled plants being the most common source of A. tumefaciens. Plants may be colonized by A. tumefaciens, but not infected until wounded. Some strains of A. tumefaciens have a broad host range while others have a very narrow host range.

Two characteristics of gall cells not shared by normal cells are the ability of gall cells to synthesize certain compounds known as opines and the ability of gall cells to proliferate in culture in the absence of plant growth hormones. It seems that an unusual ratio of plant growth hormones (auxin and cytokinin) in infected cells plays a major role in gall formation. The ability of A. tumefaciens to produce galls requires the presence in the bacterium of a very large plasmid (Ti plasmid). A small region of DNA (T-DNA) in the Ti plasmid is transferred into wounded plant cells, and becomes integrated into the plant's chromosomal DNA. Expression of genes within the T-DNA results in an alteration of the plant cells' hormonal balance, causing the crown gall growth. This "natural genetic engineering" is the basis for the technique used in this request to incorporate foreign genes into plants.

5.3.1 Biochemical Genetics of T-DNA

Ti plasmids have the unique natural ability to transform cells of susceptible host plants by the insertion of an 8 to 23 kilobase (kb) sector of plasmid DNA into host chromosomal DNA. This transferred DNA (T-DNA) causes the transformed cells to synthesize new metabolites called opines, with the synthase

enzymes for opine production encoded by T-DNA genes. Additional genes in T$_i$ DNA affect the phytohormone balance of transformed cells as evidenced by altered morphology of crown gall cells transformed by various mutant T-DNAs. Transformed plant cells containing wild type T-DNA grow in vitro without an exogenous supply of either auxin or cytokinin, whereas normal plant cells usually require both substances for growth in tissue culture. Mutations in one T-DNA locus result in callus cultures from which abundant roots proliferate ("rooty" mutants), while mutations in a second T-DNA locus result in callus cultures from which shoots proliferate ("shooty" mutants). Transformation of tobacco cells by shooty mutant T-DNA results in callus cultures which exhibit a complex auxin requirement. Such tissues will grow on hormone free medium under conditions which allow shoot proliferation, but otherwise require auxin for continuous growth. The rooty and shooty functions are coded in a T-DNA region common to octopine, nopaline, "unusual nopaline" and agropine Ti plasmids of wide host range, evidence that all such Ti plasmids promote crown gall growth of plant cells through a common mechanism.

T-DNA acts as a natural gene vector for A. tumefaciens, producing transformed plant cells that display an abnormal hormonal balance and synthesize new metabolites. Recent work has shown that the Ti plasmid can be exploited as an artificial gene vector to introduce novel genes into plant tumor cells. Heretofore, a major obstacle to the exploitation of Ti plasmids as

gene vectors for higher plants has been the difficulty of regeneration of whole plants from transformed plant cell lines. Cloned gall-forming lines of tobacco cells containing wild type nopaline plasmid pTiT37 T-DNA spontaneously, or upon cytokinin induction, regenerated shoots that displayed varying degrees of normalcy upon grafting onto healthy host plants. Such shoots synthesized nopaline, failed to form roots, and were resistant to superinfection by A. tumefaciens. When fertile, these grafted shoots produced seed that gave rise to normal plants that lacked nopaline, produced roots and were sensitive to A. tumefaciens, as well as seeds that would not produce intact plants. The normal plants did not contain T-DNA due to Mendelian segregation of the T-DNA. Similar results have been obtained in studies of tobacco cells transformed in vitro by either octopine or nopaline type T-DNA. In such experiments, opine-positive plant cells with roots were not obtained, and shoots obtained by grafting were usually both opine-positive and resistant to superinfection by A. tumefaciens. One example has been reported of opine-positive complete plants regenerated from a crown gall tumor initially incited by a shooty mutant of octopine T-DNA. These plants segregated the octopine trait in Mendelian fashion to healthy progeny, evidence that T-DNA was situated in chromosomes of the parental tissue. However, the T-DNA in these plants was found not to be full length. A large deletion of the central part of T-DNA apparently gave rise to plant cells with little T-DNA except for the octopine synthase gene. In experiments conducted by Agracetus it has been shown that it is possible to remove

virulence of Agrobacterium by "destruction" of one specific gene within the T-DNA, providing a means of transporting new genes into healthy plants. Specifically the destruction of the cytokinin gene within the T-DNA was accomplished by insertion into that gene of the genes encoding yeast (Saccharomyces cerevisiae) alcohol dehydrogenase I (ADH) and the bacterial aminoglycoside phosphotransferase gene from Tn*5* which encodes for kanamycin resistance. This genetically engineered T DNA is called ADH-T-DNA.

5.3.2 Reproduction of the Plants

The plants produced from the tissue culture (these plants are called HADH2) were then grown to sexual maturity, were allowed to flower, and were self-pollinated. Seeds were set and germinated. Many of the resulting seedlings tested produced nopaline, although the relative levels varied. An analysis of the DNA of the R1 progeny, using Southern hybridization, confirmed that the entire T-DNA was contained in the plants, although the copy number appeared to vary and occasional rearrangements of some T-DNA was observed. Reproduction by self-fertilization has been accomplished through more than four generations, and outcrosses have been conducted with commercial tobacco varieties. The T-DNA has been retained throughout these crosses as indicated by nopaline presence.

Despite the occasional rearrangements in restriction patterns of the novel DNA in these plants, there have been no apparent morphological changes which would suggest a reversion of the altered T-DNA to its ability to form crown gall (this would presumably result in gall growth in a localized region of the plant, although such a gall would not be capable of passage to subsequent generations). Agracetus scientists have observed no deleterious effects of the T-DNA presence, but have instead found potential benefits.

5.3.3 Verification of Disease Resistance

Agracetus scientists have found that the plants containing ADH-T-DNA are resistant to infection by various Agrobacterium strains. In the initial greenhouse experiments, ten mature R1 plants were selected from the HADH2 plants. Ten control plants from the H425 line from which the HADH2 plants were derived were also selected. All plants were mature and apparently in good health. Five plants of the HADH2 and the H425 were each inoculated with A. tumefaciens strain A208 (containing a wild type, gall-forming Ti plasmid), and five of each of the two lines of plants were inoculated with a different A. tumefaciens, strain B6-806. The inoculations were made on each side of a low portion of the stem of each plant. The inoculations were made with 1 ml of concentrated bacterial culture to superinfect the plants. The plants were thoroughly wounded in and about the inoculation site with multiple punctures. After 27 days of continued growth, all

the H425 plants of both groups had developed crown gall growth. None of the HADH2 plants of either group had developed any perceptible crown gall growth at all.

5.4 Possible Effects of the Proposed Field Test

5.4.1. Accidental Escape of the Genetically Engineered Plants from the Test Plot

Unlike animals which have the ability to walk and to climb, or microorganisms which can be carried by the wind, tobacco plants are rooted in soil and thus can not accidentally escape from a test site. Therefore effects of this field test will be confined to the small test plot.

5.4.2. Escape of Pollen or Seeds of the Genetically Engineered Plants from the Test Plot

As noted above all but 5 of the plants will be harvested prior to flowering. Five plants will be kept in the field until maturity for seed collection. Agracetus personnel will bag the flowers of these five plants to prevent dispersal of their pollen. Thus no pollen or seeds should escape. The area of the field test is over 10 miles from any other tobacco. One-fourth mile (80 rods) is considered sufficient isolation to prevent uncontrolled pollination in the production of certified tobacco seed (15). Nicotiana alata is sometimes grown in home flower gardens as an ornamental. It is

difficult to set seed on this species with N. tabacum pollen, even with hand pollination. Usually any seed set fails to germinate; when it does, the hybrids are sterile (17). Thus perpetuation of the genetic alteration by transfer of pollen outside the experimental plot is extremely unlikely. Unrelated plant species cannot be pollinated by tobacco pollen.

5.4.3. Excision of the Inserted Recombinant DNA from the Genetically Engineered Plants

Several hundred healthy tobacco plants with ADH-T DNA (HADH2 plants), over the past two years, have been carefully examined by Agracetus' scientists, consultants and technicians. There has been no instance of any genetically engineered plant spontaneously exhibiting a crown gall. Almost all of these plants have been observed from the seedling stage through development of mature seeds. Since the crown gall phenotype requires cytokinin gene activity, and the cytokinin gene is inactivated by the foreign DNA insertion, this is the result one would expect.

If a precise excision of the gene encoding yeast alcohol dehydrogenase I and the bacterial aminoglycoside phosphotransferase gene occurs with concomitant reconstitution of an active cytokinin gene in the T-DNA, then the result would be a T-DNA that is found in a normal crown gall. If such an excision and ligation would occur in an HADH2 plant cell, then one might expect a crown gall to appear from the site of that

cell. The probability of such an occurrence (precise excision and ligation) theoretically is expected to be very rare. As mentioned above, Agracetus scientists have not seen any gall spontaneously appearing in at least a thousand HADH2 tobacco plants examined over the past two years. If one assumes that a plant contains 100 million cells and Agracetus scientists have looked at 1,000 plants, then one may estimate that the chance of a precise excision occurring is less than one in 100 billion cells.

However, if one assumes that an exact excision will occur at some finite frequency (perhaps in one cell from one thousand plants), it is possible that a crown gall might appear from that cell. The only symptom that should result would be a single crown gall. There is no reason that such a crown gall will spread to other sites on the same plant. No Ti plasmid virulence genes are present. Unlike a true crown gall, no Agrobacterium cells are present to initiate new galls in the areas of the plant that may become injured. Crown galls that Agracetus scientists have induced (by inoculating Agrobacterium into injured sites) in non-genetically engineered tobacco lines have, in no case, spread to other sites on the same plant. It is even more unlikely that the crown gall will spread spontaneously to another susceptible plant. In Agracetus' crowded greenhouses, where crown gall infected plants have been randomly spaced among plants susceptible to crown gall, there has been no case of a crown gall appearing in a plant not intentionally infected (by wounding) with Agrobacterium. Many experiments by other scientists cited in the published scientific literature confirm

such restriction of symptoms to inoculated plants.

The bacterial aminoglycoside phosphotransferase gene is expressed in Agrobacterium but not in the genetically engineered plants. The yeast ADH gene is not expressed in Agrobacterium or in the genetically engineered plants. If, somehow, the yeast ADH gene or the bacterial aminoglycoside phosphotransferase gene with or without T-DNA would be integrated into another organism it is extremely difficult to imagine how any type of problem might occur.

5.4.4 Alterations in Palatability to Insect Pests

Major insect pests of tobacco in Wisconsin are green peach aphids and green hornworms (22). Many other insect species do not colonize tobacco. Any change in the chemical constituents of the plant, however, might theoretically alter the response of insects so that the plants would provide an opportunity for their increase not possessed by the parent variety. The experimental plot will be monitored frequently to detect any abnormal insect populations. Approved insecticides will be applied if necessary. Any unusual insect populations would be ecologically significant only if the experimental tobacco were propagated extensively in the environment. This will not be the case and any environmental impact should be limited to the test plot.

5.4.5 Alterations in Palatability to Nematodes

A number of species of nematodes feed on tobacco roots (18). Of the species that occur in Wisconsin, the meadow nematode (Pratylenchus sp.) can be damaging when tobacco is planted after sod. This ectoparasitic nematode does not maintain itself well on tobacco, however, and is not an important pathogen on a crop continuously cultivated in the same field. In monitoring nematode populations, roots and rhizosphere soil will be collected, the nematodes screened, counted, and identification made. Any effect of the experimental tobacco on nematode populations would not be significant beyond the tobacco test plot, due to the relative immobility of the nematodes. Such an effect would not be significant subsequently in the absence of tobacco because the population would decline in the absence of the selective host. The root knot nematode is not a factor in Wisconsin tobacco production, but its possible presence in the experimental tobacco will be monitored at intervals during the growing season. Roots will be dug and examined for the presence of nematode galls.

5.4.6 Alterations in Susceptibility to Fungal Diseases

A number of fungus diseases are important in the cultivation of tobacco. There is no reason to believe that the genetic changes introduced into the tobacco in the test plot should effect susceptibility to fungal diseases. Any changes should be confined to the test plot.

5.4.6.1 Wisconsin tobacco varieties are moderately resistant to the fungus causing black root rot (Thielaviopsis basicola) (30). A susceptible variety planted in soil that previously produced tobacco is very markedly stunted. The fungus is nearly ubiquitous in Wisconsin soils, so that susceptible tobacco varieties are self-eliminating. Havana 425 has this resistance; whether it is retained in the experimental tobacco is undetermined.

5.4.6.2 Tobacco anthracnose, caused by Colletotrichum destructivum, occurs occasionally on plants in the seed bed. Commercial varieties, including Havana 425, are susceptible, but infection is rare.

5.4.6.3 Blue mold, caused by Peronospora tabacina, has recently been a devastating disease in the Eastern United States and Canada. The fungus does not remain viable over winter in Wisconsin or many parts of the United States. Although the disease occurred once in Wisconsin, (14) the state has remained free of it since, presumably because the winds during the growing season are predominantly western and southwestern. Spores produced by the perennating fungus in the Caribbean region are extremely unlikely to reach Wisconsin.

5.4.6.4 There are a number of other fungus diseases of tobacco that have not been recorded in Wisconsin. The absence of these diseases is a result of the absence of the pathogens or conditions unfavorable for disease development, not because of the resistance of the tobacco. Some of the more important of these are (18):

Fusarium wilt (Fusarium sp.)
Frog eye (Cercospora nicotianae)
Brown spot (Alternaria sp.)
Black shank (Phytophthora nicotianae f. parasitica)

5.4.7 Alterations in Susceptibility to Bacterial Diseases

There are two bacterial diseases that are, at times, important in tobacco culture in Wisconsin. The experimental tobacco to be field tested has been altered to make it insusceptible to crown gall. This disease, however, is not known to occur naturally on field tobacco, so that resistance to the disease would have, per se, no ecological effect. There is no reason to believe that the genetic changes introduced into the tobacco in the test plot should affect susceptibility to bacterial diseases other than crown gall. Any changes should be confined to the test plot.

5.4.7.1 Wildfire, caused by Pseudomonas syringae pv. tabaci, may be particularly damaging in seed beds and following storms in field tobacco. One Havana Seed variety (Havana 503) was released to growers a number of years ago (24) as being resistant to this disease. Subsequently, a second race (race 1) of the bacterium appeared that was pathogenic to Havana 503. A new variety of tobacco, resistant to both races of P. s. pv. tabaci, is in the process of being released. This resistance is the result of transfer of genetic material from N. rustica to N. tabacum (25).

5.4.7.2 Blackfire, caused by P. s. pv. angulatum, may damage field grown tobacco. The causal organism is closely related to P. s. pv. tabaci and varieties resistant to one organism are resistant to the other (9). Havana 425 and the experimental tobacco to be field tested are susceptible to both these bacterial diseases.

5.4.8 Alterations in Susceptibility to Virus Attack

A number of virus diseases occur in tobacco in Wisconsin. There is no reason to believe that the genetic changes introduced into the tobacco in the test plot should affect susceptibility to viral diseases. Any changes should be confined to the test plot.

5.4.8.1 Of more importance formerly than now is tobacco mosaic virus. Most (over 90 percent) of the tobacco grown in Wisconsin now is resistant to this virus by reason of its hypersensitive reaction. The experimental tobacco to be field tested was derived from a variety resistant to tobacco mosaic virus and it retains this resistance.

5.4.8.2 Cucumber mosaic virus has infected a large proportion of the plants in certain fields. Symptoms are very similar to those of tobacco mosaic virus infection. There is no satisfactory resistance to the virus in the genus Nicotiana (26). The virus is transmitted by aphids from infected perennial plants or from species in which the virus is seed transmitted.

5.4.8.3 Other viruses occur in low incidence in Wisconsin tobacco. Havana 425 tobacco is considered to have resistance to tobacco veinmottling virus (26), but this virus has not been reported to occur in Wisconsin.

5.4.9 Alterations in "Weediness" (Persistence of Tobacco)

In the unlikely event of undesirable effects accompanying cultivation of the genetically engineered tobacco, it may be relevant to consider whether this tobacco might persist without cultivation, although no member of the genus Nicotiana is known

to occur as a problem weed. In the cultivation of Wisconsin tobacco, the tops of the plants are broken off at the time flowers begin to open. Subsequent growth from buds at leaf axils is prevented chemically, or the shoots are removed by hand. Thus no seed is produced on plants grown for their leaves. It is possible that seed might be produced on "suckers" which sprout from stumps left after harvest if a killing frost does not occur until late in the fall. In the event that a killing frost has not occurred by the time that suckers begin to initiate flowering, the plants will be killed by use of herbicides.

5.4.10 Pilfering from the Experimental Plot

Pilfering from experimental plots has been known to occur in conventional plant breeding trials. It seems unlikely that this will occur in the present trial for the following reasons:

a. The experimental plants have no visible quality that would suggest to a grower that they would be more desirable than commercially available varieties. In appearance in the greenhouse they are not detectably different from the unaltered Havana 425 parent. It is, of course, much to the advantage of Agracetus that none of the engineered tobacco fall into unauthorized hands.

b. The plot will be located in an area under the control of Agracetus and not in an area of commercial tobacco production.

c. Agracetus will not disclose publicly the plot location, nor will it label the area in such a way that anyone unfamiliar with the trial could determine that any of the plants were genetically altered. Location of the experimental tobacco within the plot will be a confidential record with Agracetus and will not be indicated in the plot area.

5.5 Effect of Alternatives

5.5.1 Approval of Proposal of May 5, 1983

The original proposal of May 5, 1983, was more extensive than that considered in Section 4.1 in that the field test would include cotton, soybean, corn, tomato and potato in addition to tobacco. Expanding the tests to additional crops would increase the number of test sites needed which would also increase the possibility for the release of pollen from genetically engineered plants. In addition, the logistics of monitoring test sites for six crops would be more difficult.

5.5.2 Approval of Proposal of August 19, 1983

This proposal was more extensive than that considered in Section 4.1 in that the field test would include cotton and tomato in addition to tobacco. Expanding the tests to additional crops would increase the number of test sites needed which would also increase the possibility for the release of pollen from genetically engineered plants. In addition, the logistics of monitoring test sites for three crops would be more difficult.

5.5.3 Withhold Approval

Withholding approval may have adverse ecological consequences in the long run. Agricultural crops are constantly under attack by a wide variety of pests and adverse climatological factors. Current defenses against these predations are imperfect inasmuch as significant portions of world crop production are lost every year. In addition, the defenses themselves often have adverse ecological effects since large quantities of often toxic chemicals are used for pest control.

If this approval is withheld, researchers could still use classic methods of plant breeding. These methods do not seem as capable as recombinant DNA techniques of producing dramatic improvements in resistance of plants to heat, drought, pests and other environmental factors. Farmers would probably continue widespread use of pesticides to control diseases and pests. Many

of these chemicals have known adverse health and environmental effects.

An additional effect of this alternative is to retard scientific progress. Agracetus has developed a genetically engineered tobacco plant as a precursor for more sophisticated genetically engineered plants which may have significant benefit to agriculture. Withholding approval of this proposal will delay the realization of these possible benefits as well as impede the advance of science.

5.6 Summary and Conclusions

The NIH concludes that no significant environmental effects will result from conduct of the proposed field test described in Section 4.1. Any ecological effects which could possibly be attributed to this field test should be limited to the small 70' x 80' test plot during the time period the test is underway. Due to the non-pathogenicity of the T-DNA sequence, the inherent risk is minimal. Safety factors have been added to the test design to minimize the possibility of adverse ecological effects. One safety factor is the small test plot size (0.05 acres) and its isolation (over 10 miles) from the nearest conventional tobacco plot. Another safety factor is that, except for five plants, test personnel will harvest all plants prior to flowering. The remaining five plants will be bagged prior to flowering to avoid pollen dispersal. This proposed field test will not have a significant ecological effect on living creatures or their environment. Should unanticipated effects arise, the isolation of the test site and manner

of conducting the test indicate that the effects can be readily contained and would have no permanent effect on the environment.

6.0 CONSULTATION

Those consulted in preparation of this environmental assessment were:

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Dr. Jerome Miksche, Department of Agriculture

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Dr. Milton Zaitlin, Professor of Plant Pathology,

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Figure 1

FIELD PLOT FOR FIELD TESTING GENETICALLY ENGINEERED HAVANA TOBACCO

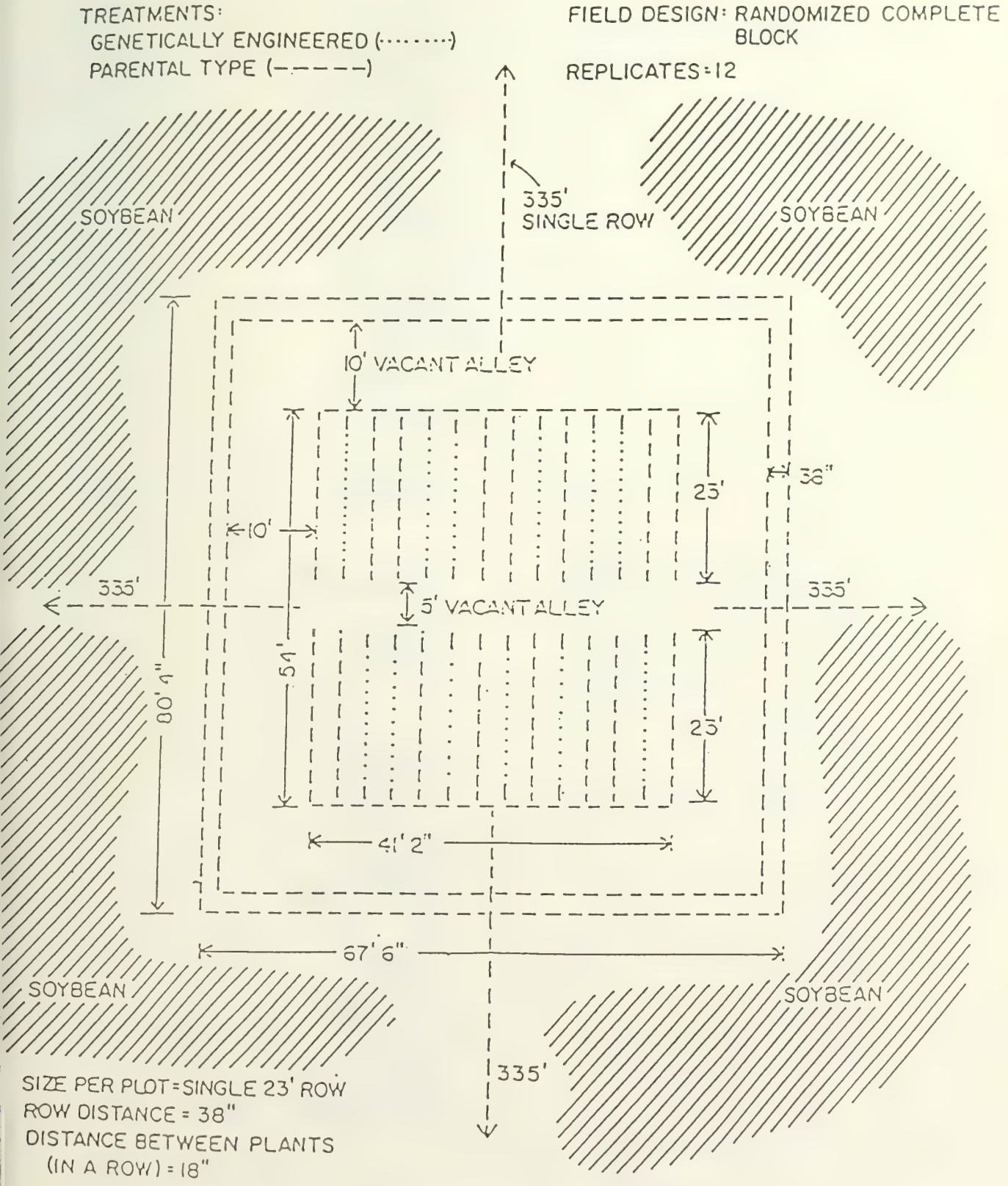
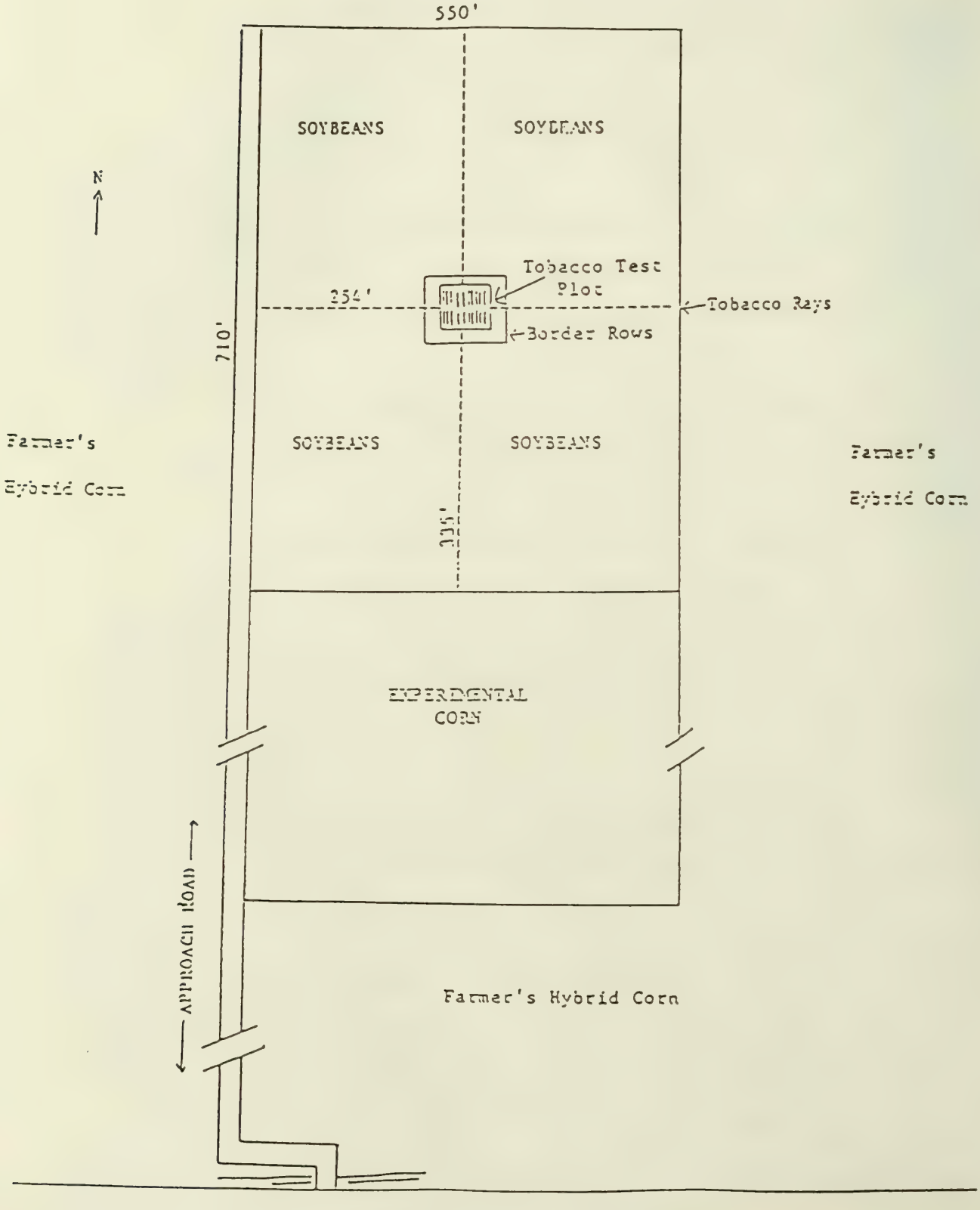


Figure 2

AGRACETUS EXPERIMENTAL FARM (20 ACRES)



Friday
November 22, 1985

Part VI

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines; Notice

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health (NIH), PHS, DHHS.

ACTION: Notice of actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth four actions taken by the Director, NIH, under the November 1984 NIH Guidelines for Research Involving Recombinant DNA Molecules (49 FR 46266).

EFFECTIVE DATE: November 22, 1985.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20892, (301) 496-6051.

SUPPLEMENTARY INFORMATION: Today four actions are being promulgated under the NIH Guidelines for Research Involving Recombinant DNA Molecules. Three of the proposed actions were published for comment in the Federal Register of August 19, 1985 (50 FR 33462), and reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on September 23, 1985. The fourth action was published in the Federal Register of March 28, 1985 (50 FR 12456). It was considered and tabled at the May 3, 1985, RAC meeting; it was rereviewed and recommended for approval at the September 25, 1985, RAC meeting.

In accordance with Section IV-C-1-b of the NIH Guidelines, these actions have been found to comply with the NIH Guidelines and to present no significant risk to health or the environment.

Part I of this announcement provides background information on the actions. Part II provides a summary of the actions of the Director, NIH.

I. Decision on Actions Under NIH Guidelines

A. Request for Permission to Clone Shiga-Like Toxin From the Families Enterobacteriaceae and Vibrionaceae in E. coli K-12

Dr. Alison O'Brien of the Uniformed Services University of the Health Sciences (USUHS) had previously (49 FR 36052) received permission from the National Institutes of Health to clone the structural gene of the Shiga-like toxin (SLT) of *Escherichia coli* in *E. coli K-12*.

Subsequently in a letter of July 26, Dr. O'Brien requested permission to clone SLT structural genes, defined either by nucleotide sequence homology with SLT gene probes from *E. coli* or by antigenic cross-reactivity of their gene products with purified *E. coli* SLT, from bacterial species classified in the families *Enterobacteriaceae* or *Vibrionaceae* into *E. coli* K-12 under conditions similar to those specified in the previous permission. Dr. O'Brien requested premission to broaden the scope of study of SLT toxin for the following reasons: (1) To investigate the potential role of SLT in the pathogenicity of a variety of bacteria; (2) to compare the SLT genes from various pathogenic bacteria; and (3) to facilitate the development of an experimental *Vibrio cholerae* vaccine strain deleted for SLT genes.

This proposal was published in the August 19, 1985, Federal Register for public comment. No cumments were received during the comment period.

On August 16, 1985, the RAC Working Group on Toxins considered this proposal. A portion of the minutes of th meeting pertaining to this proposal reads as follows:

"Dr. Gottesman said Dr. Alison O'Brien of the Uniformed Services University of the Health Sciences (USUHS) was requesting permission to clone SLT structural genes (defined either by nucleotide sequence homology with SLT gene probes from *E. coli* or by antigenic cross-reactivity of their gene products with purified *E. coli* SLT) from bacterial species classified in the families *Enterobacteriaceae* or *Vibrionaceae* according to Bergey s Manual of Systematic Bacteriology into *E. coli* K-12 under the conditions specified in Federal Register, Volume 49, Number 179. Dr. O'Brien had previously sought and had obtained permission from the NIH to clone SLT from *E. coli* in *E. coli* K-12 host-vector systems. The language of that permission appeared in Federal Register 49, Number 179, and was incorporated into the NIH Guidelines as Appendix F-IV-H

"Dr. Gottesman said Appendix F-IV-H of the NIH Guidelines currently reads.

'The intact structural gene(s) of the Shiga-like toxin from *E. coli* may be cloned in *E. coli* K-12 under BL3+EK1 containment conditions.

'*E. coli* host vector systems expressing the Shiga-like toxin gene may be moved from BL3 to BL2 containment conditions provided that: (1) The amount of toxin produced by the modified host-vector systems is no greater than that produced by the positive control strain 933 *E. coli* 0157:H7, grown and measured under optimal conditions; and (2) the cloning vehicle is to be an EK1 vector preferably belonging to the class of poorly mobilizable plasmids such as pBR322, pBR328, and pBR325.

'Nontoxinogenic fragments of the Shiga-like toxin structural gene(s) may be moved from BL3+EK1 to BL2+EK1 containment conditions or such nontoxinogenic fragments may be directly cloned in *E. coli* K-12 under BL2+EK1 conditions provided that the *E. coli* host-vector systems containing the fragments do not contain overlapping fragments which together would encompass the Shiga-like toxin structural gene(s).' "

". . . Dr. O'Brien said she and her group had been investigating organisms for the presence of SLT. SLT is defined as being cytotoxic to cells and antigenically cross-reactive with purified *E. coli* SLT. SLT has been identified in members of the families *Enterobacteriaceae* and the *Vibrionaceae*, and she was now requesting permission to clone SLT genes from the *Vibrionaceae* and the *Enterobacteriaceae* in *E. coli* K-12. The major goal of this work is to develop a cholera toxin vaccine.

"Dr. O'Brien suggested SLT may be a common property of these bacteria; in organisms such as *Shigella dysenteriae* 1, SLT may play a role in virulence when a large amount of the protein is produced and other virulence factors are present.

"Dr. O'Brien said SLT has also been recently identified in one strain of *Campylobacter* and two strains of *Aeromonas*. The *Aeromonas* are classified as *Vibrionaceae*. The *Campylobacter* had at one time been classified by Bergey's Manual as *Vibrionaceae* but had recently been reclassified as an orphan species.

"Dr. O'Brien pointed out that a number of *Campylobacter* characteristics are common to the *Vibrionaceae*. She asked whether she might receive permission to clone SLT genes from *Campylobacter*.

"Dr. Milewski said the August 19, 1985, Federal Register announcement (50 FR 33462) published 30 days before the RAC meeting and describing the proposal stated that Dr. O'Brien requested permission to clone the SLT gene from members of the families *Enterbacteriaceae* and *Vibrionaceae* . . . She suggested the working group first vote on the proposal as it appeared in the Federal Register. The group could subsequently offer a recommendation on experiments involving *Campylobacter*.

"Dr. Levine pointed out that investigation in other parts of the world may perform the types of experiments

requested by Dr. O'Brien under much less restrictive conditions. He though U.S. investigators are unfairly shackled by differences in rules on the cloning of toxin genes.

"Dr. Gottesman said there are two approaches to dealing with differences in rules between countries: (1) The working group may suggest the NIH Guidelines be modified; and (2) the investigator may request more generic approvals.

"Dr. Gottesman suggested the working group proceed by voting on those portions of request which were published in the August 19, 1985, Federal Register and subsequently propose and vote on other actions the working group deems reasonable.

"Dr. Gottesman asked whether the SLT structural gene is carried on a phage. Dr. O'Brien replied that high toxin production in bacteria usually appears to be associated with the presence of SLT converting phages such as 933J and H19A/J but not always. A low level of toxin production can consistently be detected in the absence of phage, and may be associated with a chromosomal gene. Dr. O'Brien hypothesized that the phage gene may be a variant of the chromosomal gene. She suggested other variants of the gene might be found in other bacterial species. She added that the Shiga toxin gene appears to be a chromosomal gene in *Shigella dysenteriae* 1 since the phage cannot be induced.

"Dr. Gottesman asked what is known about the host range of the SLT converting phages. Dr. John Newland of USUHS said the phages can infect other *E. coli* strains; other bacterial families have not been studied to determine whether these phages will infect members of these families.

"Dr. Gottesman asked how much toxin was produced when the SLT gene under its own regulation on a multicopy plasmid was introduced into the *E. coli* host-vector system. Dr. O'Brien replied that a seven fold increase in toxin production compared to the standard strain 933 *E. coli* 0157:H7 occurs when the SLT gene under its own regulation is introduced into the *E. coli* host-vector system.

"Dr. O'Brien said the issue of increased SLT production by the *E. coli* host-vector system should be kept in perspective. Under optimal laboratory conditions, *Shigella dysenteriae* 1 strain 60R produces 10^6 cytotoxic toxin doses per milligram (mg) protein . . .; the standard strain 933 *E. coli* 0157:H7 produces 10^7 cytotoxic doses (CDs) per mg protein under optimal conditions. The *E. coli* host-vector strain carrying the SLT gene on a multisopy plasmid produces $7x10^7$ CDs per mg protein under optimal conditions. This level of toxin production is still less than that produced by the *Shigella dysenteriae* 1 strain. She added that *Shigella flexneri* produces approximately 10^4 CDs per mg protein under optimal conditions. The *Vibrio cholerae* vaccine strain produces 10^4 CDs per mg protein under optimal conditions.

"Dr. Collier pointed out that at this time the specific activity of SLT toxin is not known. Dr. Gottesman said arguments on mechanism of entry into the animal and treatability of symptoms support a recommendation to RAC that this proposal be recommended to the NIH Director. Dr. Formal said *E. coli* host-vector systems offer an additional safety factor since experience has shown *E. coli* K-12 will not ordinarily colonize the bowel.

"Dr. Collier moved that the working group approve of Dr. O'Brien's request as it appears in the August 19, 1985, Federal Register.

"Dr. Gill said he would like the record to show the working group was taking this action because it judged that *E. coli* host-vector systems carrying the SLT gene no more harmful than Shiga toxin expressing strains found in nature.

"By a vote of six in favor, none opposed, and no abstentions, the working group recommended RAC approve Dr. O'Brien's request as it appeared in the August 19, 1985, Federal Register. . . .

"Dr. Newland asked whether 933 *E. coli* 0157:H7 would be the standard against which activity is measured regardless of what strain was used as the source of the SLT gene. Dr. Gill said the strain specified in Appendix F-IV-H of the NIH Guidelines would be standard strain regardless of the source of the SLT gene.

"Dr. O'Brien asked the working group to consider substituting in Appendix F-IV-H a shigella strain which produces more toxin than 933 *E. coli* 0157:H7 as the standard against which to measure toxin production. *Shigella dysenteriae* 1 strains produce approximately 10^8 CDs mg protein in cell lysate; standard strain 933 *E. coli* 0157: H7 produces 10^7 CDs per mg protein under optimal conditions.

"Dr. Collier said the question is whether there is any reason to believe an *E. coli* K-12 host-vector strain expressing SLT or Shiga toxin on a high copy number plasmid would be any more toxic than *Shigella* found in nature. Dr. Levine pointed out that *E. coli* K-12 does not colonize the gut and a K-12 host-vector system carrying the SLT gene would not be more pathogenic than *Shigella dysenteriae*. He thought 10^8 CDs per mg protein in cell lysates would be an appropriate upper limit for permitting a clone to be removed from BL3 containment.

"Dr. Gill said an extra margin of safety is provided by the specification in Appendix F-IV-H of 10^7 CDs per mg protein in cell lysates as the upper limit for removal of SLT clones from BL3 containment.

He would prefer strain 933 *E. coli* 0157:H7 which produces 10^7 CDs under optimal containment be the standard strain for determining whether a particular host-vector strain could be removed from BL3 containment. If cloning of the Shiga toxin gene on a high copy number plasmid in *E. coli* K-12 resulted in a 10 fold increase in toxin production, a very high level of toxin, 10^8 CDs, would be produced.

"Dr. Kopecko said it was important to clone the Shiga gene from *Shigella dysenteriae* 1 in order to understand how toxin gene expression is controlled since *Shigella dysenteriae* 1, an epidemic strain, produces more toxin than other bacteria. He thought 10^8 CDs per mg protein is equivalent to the highest level of Shiga toxin expression found in nature; therefore, the working group should recommend *E. coli* K-12 host-vector systems expressing 10^8 CDs per mg protein be permitted to be moved from BL3 to lower containment. K-12 strains producing more than 10^8 CDs per mg protein will not be permitted to leave BL3 containment under the proposed language.

"Dr. Gottesman said the issue is whether *E. coli* K-12 producing high levels of Shiga toxin would be hazardous to animals.

"Dr. Habig said he did not see why *E. coli* K-12 host-vector systems carrying the Shiga toxin gene would pose a greater danger than *Shigella dysenteriae* 1. Dr. Kopecko pointed out that clinical laboratories routinely work with *Shigella dysenteriae* 1 at BL2 conditions. He did not think *E. coli* K-12 host-vector systems would be more dangerous than *Shigella dysenteriae* 1. Dr. Gottesman said she felt comfortable with permitting *E. coli* host-vector systems expressing 10^8 CDs of SLT to be used at BL2 containment since *E. coli* K-12 does not colonize the gut.

"Dr. Levine suggested a number such as 10^8 CDs be chosen as the upper limit of toxin a host-vector system might express if it is to be removed from BL3 to a lower containment level.

"Dr. Levine said *E. coli* K-12 is not an invasive pathogenic organism. Several factors are necessary for pathogenicity, and even for *Shigella* more is required than simply expression of the toxin gene. An attenuated Shiga bacillus

vaccine strain which produces as much Shiga toxin as the parent pathogenic strain but lacks invasiveness factors did not cause disease when fed to human volunteers. In the single case in which the strain reverted to invasive, the human volunteer became ill.

"Dr. Formal said he had constructed a strain by introducting half of the *Shigella flexneri* chromosome and an SLT converting phage into *E. coli* K–12. The introduced *Shigella flexneri* genes were thought to include all of the genes necessary for virulence. The constructed strain had no effect on monkeys in feeding experiments although it produced 10^7 CDs per mg protein of SLT under optimal laboratory conditions.

"Dr. Gill asked whether concern exists about transfer to other organisms of a high copy number plasmid carrying the Shiga toxin gene and its control elements.

"Dr. Newland said the probability of transfer of the SLT or Shiga toxin gene by phage in nature is higher than the probability of transfer from K–12 by poorly mobilizable plasmid vectors.

"Dr. Gottesman asked what laboratory conditions are necessary to obtain optimal expression of the SLT gene in the *E. coli* K–12 host-vector system. Dr. Newland replied that antibiotic pressure is necessary. In addition, several other procedures such as using specially treated medium are also required. If an SLT clone is simply cultured in the conditions usually used to culture the host-vector system, toxin production is generally a factor of 10^2 lower than the highest level of expression obtained under optimal conditions with that clone.

"Dr. Newland said he had observed that the recombinant plasmid carrying the SLT gene appears to be rapidly lost from the *E. coli* host-vector system in the absence of selective antibiotic pressure. The plasmid without the SLT gene is not so rapidly lost in the absence of selective pressure. He and Dr. O'Brien hypothesized that high levels of toxin may be toxic to bacteria. The rapid loss of the recombinant plasmid in the absence of selective pressure may be an additional safety factor.

"Dr. Gottesman asked whether the working group would consider modifying Appendix F–IV–H by raising the upper level of toxin expression to 10^5 CDs per mg protein in cell lysates in exchange for an assurance that the plasmid will be rapidly lost in the absence of antibiotic pressure. Alternatively, an upper level of expression need not be fixed if the plasmid will be rapidly lost since rapid plasmid loss will provide a measure of safety.

"Dr. Levine said one problem with Dr. Gottesman's proposal is that language gerferic for experiments involving *E. coli* may not apply to experiments involving *Vibrio* or *Shigella*. A second concern is the variability of the SLT assay; without an internal standard it is difficult to know how much toxin is produced. He asked Dr. O'Brien how great a difference is observed using the same procedure from experiment to experiment. Dr. O'Brien replied that a 100 fold difference in the amount of CDs produced may be observed from experiment to experiment.

"Dr. Gill moved that Appendix F–IV–H be amended to read in part:

'E. coli host-vector systems expressing the Shiga-like toxin gene product may be moved from BL3+EK1 to BL2+EK1 containment conditions provided that: (1) The amount of toxin produced by the modified host vector systems be no greater than that produced by the positive control strain 933 *E. coli* 0157:H7, grown and measured under optimal conditions, or ten times this level if the maintenance of the plasmid carrying the gene is dependent upon growth in the presence of an antibiotic . . . .'

"Dr. Levine suggested the motion be amended to read in part:

'E. coli host-vector systems expressing the Shiga-like toxin gene product may be moved from BL3+EK1 to BL2+EK1 containment conditions provided that: (1) The amount of toxin produced by the modified host vector strain be no greater than 10^5 CDs per mg protein. . . .'

"Dr. Formal suggested the motion should name a toxin producing strain as the standard rather than citing a specific number. He suggested *Shigella dysenteriae* 1 strain 60R be used for this purpose. *Shigella dysenteriae* 1 strain 60R, a rough strain not capable of colonizing the human gut, produces about ten times more toxin than 933 *E. coli* 0157:H7. Language specifying a specific strain would provide an internal standard and permit comparison from laboratory to laboratory. Dr. Kopecko agreed.

"Dr. Levine amended his motion to require use of *Shigella dysenteriae* strain 60R as the standard strain. Dr. Formal seconded this motion.

"Dr. Gottesman seconded Dr. Gill's original motion in order to permit a vote on this motion. By a vote of two in favor, four opposed, and no abstentions, Dr. Gill's motion was refused by the working group. . . .

"The working group then voted on Dr. Levine's motion. By a vote of five in favor, one opposed, and no abstentions, the working group accepted this motion. . . .

"Dr. Kopecko then offered the following motion:

'Campylobacter species have long been recognized as members of the family *Vibrionaceae*. Recently *Campylobacter* species have been separated taxonomically into an orphan genus. Because of the similarities between *Campylobacter* and *Vibrionaceae*, the Working Group on Toxins recommends that for purposes of cloning SLT *Campylobacter* can be considered as members of the *Vibrionaceae*.'

"Dr. Levine seconded this motion.

"Dr. Gill said the most important consideration in evaluating a proposal is not the species from which the gene was originally isolated but the characteristics of the gene's product and the host-vector system. He said he would support Dr. O'Brien's request to permit cloning of the SLT gene from *Campylobacter*. He thought Dr. Kopecko's motion did not convey the idea that the gene, and the characteristics of its product are the most important considerations. He suggested a substitute motion which would not refer to the source of the gene he developed.

"Dr. Formal said although he agreed with Dr. Gill, at the present time it would be difficult to precisely define the characteristics of SLT toxin. A substitute motion would have to contain such a definition.

"Dr. Milewski said Dr. Kopecko's motion was preferable because of the way the August 19, 1985, **Federal Register** announcement had been written. She said the minutes of the working group meeting would show the group strongly supports the concept that the gene is the most important consideration.

"Dr. Levine called the vote on Dr. Kopecko's motion. By a vote of six in favor, none opposed, and no abstentions, the working group approved of the motion. . . ."

The RAC discussed Dr. O'Brien's proposal at the September 23, 1985, meeting.

By a vote of seventeen in favor, none opposed, and no abstentions the RAC recommended NIH accept the working group recommendation to approve Dr. O'Brien's request as it appeared in the August 19, 1985, **Federal Register** with *Campylobacter* species considered to be members of the *Vibrionaceae*

By a vote of seventeen in favor, none opposed, and no abstentions the RAC recommended the NIH accept the working group recommendation that Appendix F–IV–H be amended to read in part:

"*E. coli* host vector systems expressing the Shiga-like toxin gene may be moved from BL3+EK1 to BL2+EK1 containment conditions provided that: (1) The amount of toxin produced by the modified host-vector systems be no greater than that produced by the positive control strain *Shigella dysenteriae* 60R, grown and measured under optimal conditions; and (2) the cloning vehicle is to be an EK1 vector preferably belonging to the class of poorly mobilizable plasmids such as pBR322, pBR328, and pBR325."

I accept these recommendations and Appendix F-IV-H has been modified accordingly.

B. Request To Clone a Hybrid Toxin Gene in E. coli K-12

Dr. John Murphy of the University Hospital of Boston University Medical Center in a letter dated July 30, 1985, requested permission to construct a hybrid molecule in which the gene coding for interleukin-2 (IL-2) is joined to a segment of the gene encoding diphtheria toxin. The diphtheria gene toxin segment encodes the A subunit and portions of the B subunit of the toxin. The hybrid gene would be cloned in *E. coli* K-12 host-vector systems.

The goal of the project is the development and testing of novel therapeutic agents targeted at the interleukin 2 (IL-2) receptor to combat rejection and/or create a state of tolerance in organ transplantation. As a by-product, some of the agents may have therapeutic potential for patients with leukemia and lymphoma.

This proposal was published in the August 19, 1985, Federal Register for public comment. No comments were received on the proposal during the comment period.

On August 16, 1985, the RAC Working Group on Toxins considered this proposal.

Although not part of the action requested in this proposal, it should be noted that Dr. Murphy was previously granted permission (Appendix F-IV-I, 49 FR 46279) to propagate under BL4 containment a hybrid molecule composed of the gene coding for α-melanocyte stimulating hormone (α-MSH) and a segment of the diphtheria toxin gene. On August 16, 1985, the RAC Working Group on Toxins considered a request from Dr. Murphy to remove these clones from BL4 containment. Dr. Murphy submittted data on the effects on guinea pigs challenged with: (1) The viable and boiled *E. coli* K-12 host-vector system (the control); (2) the viable and boiled *E. coli* K-12 host-vector system carrying and expressing the hybrid gene; and (3) the conjugate protein. After thorough discussion, the working group recommended Dr. Murphy be permitted to proceed with experiments involving clones carrying the α-MSH-diphtheria toxin gene at BL2 containment with BL3 practices. I previously accepted this recommendation.

The portion of the minutes of the August 16, 1985, meeting of the Working Group on Toxins pertaining to Dr. Murphy's proposal to construct and clone a hybrid gene composed of the genes coding for IL-2 and portion of diphtheria toxin reads as follows:

"Dr. Gottesman said in this proposal, Dr. John Murphy of the University Hospital of the Boston University Medical Center requests permission to construct a hybrid gene composed of the gene coding for interleukin-2 (IL-2) and the *SphI* segment of the diphtheria toxin gene. The hybrid gene would be cloned in *E. coli* K-12 host-vector systems.

"The long-term goal of this research is to develop novel IL-2 receptor-targeted cytotoxic agents to combat organ rejection and to create a state of graft "tolerance" in organ transplantation. Some of the products may have therapeutic potential for treating leukemia and lymphoma.

"Dr. Gottesman said the primary difference between the α-MSH-diphtheria toxin hybrid molecule proposal and the IL-2-diphtheria toxin hybrid molecule proposal is the type of cell postulated to be sensitive to the conjugate problem.

"Dr. John Williams of Beth Israel Hospital said IL-2 receptors are found on proliferating, antigen-activated T cells and to a lesser extent on activated B cells. These cells would be the targets of the IL-2-diphtheria toxin hybrid protein. Resting or memory lymphocytes do not express IL-2 receptors. There is no evidence of IL-2 receptors on tissues outside of the lymphoid compartment.

"Dr. Williams said the goal of this project is to selectively remove activated T cells and activated B cells during a well controlled time period following organ transplantation. It is hoped the IL-2-diphtheria toxin molecule would be more selective than the immunosuppressive techniques currently in use. Experiments in mice using anti-IL-2 receptor-monoclonal antibody conjugates to suppress cells which cause rejection in cardiac transplants support this hypothesis. In these experiments, the life of the graft in mice was extended from 10-14 days to three months or more in 80 percent of the animals.

"Dr. Gill said the 'worse case' scenario he could conjecture would be that an animal's T cells and B cells would be eliminated by this hybrid protein. A long observation of test animals might be necessary before such an effect would be detected. Dr. Habig said a long observation period might also be necessary for animals treated with the α-MSH-diphtheria hybrid protein.

"Dr. Levine said the IL-2-diphtheria toxin hybrid protein could be an important therapeutic agent, but it would be a new toxin and could be harmful. He suggested containment be initially set at the BL4 level.

"Dr. Martin said the largest BL4 facility at the Frederick Cancer Research Facility is run by NIAID. Any request by Dr. Murphy for use of the facility will need to be considered by NIAID in relation to other competing requests.

"Dr. Levine said since little is known about the potential toxicity of the proposed hybrid protein, the working group approach should be conservative. If BL4 containment is not available, the experiment should be permitted under BL3 containment with a recognition that concern exists about the nature of the proposed hybrid protein.

"Dr. Gottesman suggested containment could be set at BL3 with a requirement for the use of EK2 host-vector systems. Dr. Levine said he would accept the suggestion to specify EK2 vectors; he would not accept the suggestion to specify EK2 hosts.

"Dr. Gottesman asked whether any type of risk assessment experiment could be performed which would answer basic questions about the nature of the protein.

"Dr. Gill said risk assessment experiments with this hybrid protein would differ from previous risk assessment protocols because acute lethality is not a likely outcome. Tests would have to be devised to look for chronic impairment of the immune system. This type of data and the protocols necessary to generate it are different from that generally required for toxins.

"Dr. Gill offered a motion to recommend permission to proceed under BL3 conditions; EK2 vectors would be used. Dr. Levine said he would prefer the language of the motion specified the use of 'poorly mobilizable plasmid vectors such as the EK2 certified plasmids.' Dr. Gill agreed to this modification. Dr. Levine seconded the motion.

"By a vote of five in favor, none opposed, and no abstentions, the working group agreed this recommendation should be forwarded to the RAC."

The RAC discussed this proposal at the September 23, 1985, meeting.

The RAC considered the minutes of the August 16, 1985, meeting of the Working Group on Toxins and the advice of two *ad hoc* consultants, Dr. John Collier of Harvard Medical School and Dr. Thomas Waldmann of the National Cancer Institute. By a vote of twelve in favor, none opposed, and four abstentions, the RAC recommended the experiments be permitted at BL2 containment plus BL3 practices with the use of poorly mobilizable plasmid vectors such as EK2 certified plasmids. I accept this recommendation and appropriate language has been added to Appendix F of the NIH Guidelines.

C. Proposed Amendments of Appendix C–III of the NIH Guidelines

BioTechnica International, Inc., of Cambridge, Massachusetts, in a letter of August 13, 1985, proposed the following amendments to Appendix C–III of the NIH Guidelines. The first paragraph of Appendix C–III would be amended to read as follows:

"Experiments which use *Saccharomyces cerevisiae* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines."

This amendment would extend the current exemption to include strains other than "laboratory strains."

A new second paragraph would be added to Appendix C–III to read as follows:

"Experiments which use *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines."

This proposal was published in the August 19, 1985, Federal Register for public comment. No comments were received during the comment period.

The RAC considered this proposal at the September 23, 1985, meeting. It was noted that no industrial or wild *S. cerevisiae* strains are known to be pathogenic to either man or animals. In fact, products containing *S. cerevisiae*, including beer, wine, brewer's yeast tablets, bread, and yeast extract flavor enhancers, have been consumed by humans for thousands of years. Brewer's and distiller's yeasts are also consumed by the ton by domestic livestock.

Laboratory and non-laboratory strains are virtually identical with respect to their morphology, biochemistry, and growth habits. The arrangement of the genomes (i.e., the genetic maps) of the two classes are largely indistinguishable. These similarities are not surprising given that all laboratory strains were ultimately derived from wild and industrial yeasts.

Most brewing strains sporulate poorly, and the few spores that can be obtained have extremely poor viability. Thus, industrial strains tend to be much less capable of mating than laboratory strains, many of which have been bred and selected for increased ability to mate, sporulate and germinate. These factors together lead to a considerable phenotypic stability for most industrial strains that, in fact, make them desirable hosts for recombinant DNA experiments.

Evidence was presented on the great similarity between *S. cerevisiae* and *S. uvarum*.

The RAC accepted these arguments and by vote of seventeen in favor, none opposed, and no abstentions, recommended approval of the proposal as it appeared in the August 19, 1985, Federal Register.

I accept this recommendation and Appendix C–III has been modified.

D. Proposed Amendment of Part III of the NIH Guidelines

In a memorandum dated February 12, 1985, Dr. Bernard Talbot, Deputy Director of the National Institute of Allergy and Infectious Diseases, noted that under the NIH Guidelines for Research Involving Recombinant DNA Molecules certain proposals are received by NIH for review by the NIH Recombinant DNA Advisory Committee (RAC) and subsequent NIH approval.

Recently, other Federal agencies have made steps toward assuming new roles in review of recombinant DNA proposals. Because of these developments, it could now happen that a proposal submitted to the NIH for RAC review and NIH approval may be also submitted to another Federal agency for review.

Dr. Talbot stated:

"In such a case, I believe it would be advantageous for NIH to have the option of deferring to the review and approval by the other Federal agency rather than always going through review and approval by both the other Federal agency and the NIH. In order to allow this, I request that the following proposed change in the NIH Guidelines be issued for public comment, and placed on the agenda of the next RAC meeting. I propose that a new sentence be added at the end of Section III–A of the Guidelines ('Experiments that Require RAC Review and NIH and IBC Approval Before Initiation') just before Section III–A–1 of the Guidelines, as follows: 'If experiments in this category are submitted for review to another Federal agency, the submitter shall notify ORDA; ORDA may then determine that such review serves the same purpose, and based on that determination, notify the submitter that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal Agency.' "

The proposal was published in the March 28, 1985, Federal Register (50 FR 12456) for comment.

One comment was received on the proposal. Dr. Carol Lax Gronbeck of Genentech, Inc., of South San Francisco, California, wrote:

"At the present time, the Office of Recombinant DNA Activities and the NIH Recombinant DNA Advisory Committee represent the only formal government body with the expertise and large base of experience to evaluate research proposals. Genentech agrees that dual review of proposals would seem unnecessary; however, concern also exists that at this time, various Federal agencies presented with RDNA proposals may not have all of the necessary expertise and pertinent experience in-house to thoroughly review the proposals. Since the function of such review includes determination that the experiment will not adversely affect man and/or the environment, it is especially important that the appropriate experts evaluate the information. To receive proper evaluation, and until such time that Federal agencies have in place a biotechnology review policy such as that proposed by the Office of Science and Technology Policy, Genentech suggests a revision of Dr. Talbot's amendment to read as follows:

'If experiments in this category are submitted for review to another Federal agency, the submitter shall notify ORDA and may provide information regarding the necessity of dual review; ORDA may then determine if dual review is necessary based upon whether or not the reviews serve the same purpose and whether the other Federal agency has the appropriate expertise and experience to thoroughly review the proposals in a timely manner, and. . .'

"Genentech believes that RAC should enjoy the privilege of exercising an option to defer review, and it is our belief that this revision to the amendment still permits RAC the desired option, while continuing to provide constructive advice to those conducting research and protection of the public and environment."

The proposal was first considered by the RAC at the May 3, 1985, meeting. At that meeting, RAC voted to table the proposal by a vote of seventeen in favor,

none opposed, and no abstentions. Dr. Talbot's proposal was again considered by the RAC at the September 23, 1985, meeting. By a vote of seventeen in favor, none opposed, and no abstentions, the RAC recommended the NIH accept this proposal. I accept this recommendation and Section III–A has been modified.

II. Summary of Actions

A. Amendment of Section F–IV–H of the NIH Guidelines

Appendix F–IV–H has been modified to read as follows:

"Appendix F–IV–H. The intact structural gene(s) of the Shiga-like toxin from bacterial species classified in the families *Enterobacteriaceae* or *Vibronaceae* including *Campylobacter* species may be cloned in *E. coli* K–12 under BL3+EK1 containment conditions.

"*E. coli* host-vector systems expressing the Shiga-like toxin gene product may be moved from BL3+EK1 to BL2+EK1 containment conditions provided that: (1) the amount of toxin produced by the modified host-vector systems be no greater than that produced by the positive control strain *Shigella dysenteriae* 60R, grown, and measured under optimal conditions; and (2) the cloning vehicle is to be an EK1 vector preferably belonging to the class of poorly mobilizable plasmids such as pBR322, pBR328, and pBR325.

"Nontoxinogenic fragments of the Shiga-like toxin structural gene(s) may be moved from BL3+EK1 to BL2+EK1 containment conditions or such nontoxic fragments may be directly cloned in *E. coli* K–12 under BL2+EK1 conditions provided that the *E. coli* host-vector systems containing the fragments do not contain overlapping fragments which together would encompass the Shiga-like toxin structural gene(s)."

B. Addition of a New Appendix F–IV–K to the NIH Guidelines

A new Appendix F–IV–K is added to the NIH Guidelines as follows:

"A hybrid gene in which the gene coding for interleukin 2 (IL–2) is joined to a specified segment of the gene encoding diphtheria toxin may be propagated in *E. coli* K–12 host-vector systems under BL2 containment plus BL3 practices, with the use of poorly mobilizable plasmid vectors such as EK2 certified plasmids."

C. Amendment of Appendix C–III of the NIH Guidelines

The first paragraph of Appendix C–III is amended to read as follows:

"Experiments which use *Saccharomyces cerevisiae* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines."

A new second paragraph is added to Appendix C–III to read as follows:

"Experiments which use *Saccharomyces uvarum* host-vector systems, with the exception of experiments listed below, are exempt from these Guidelines."

D. Modification of Section III–A of the NIH Guidelines

A new sentence is added to the end of Section III–A of the NIH Guidelines ("Experiments that Require RAC Review and NIH and IBC Approval Before Initiation") just before Section III–A–1 of the NIH Guidelines as follows:

"If experiments in this category are submitted for review to another Federal agency, the submitter shall notify ORDA; ORDA may then determine that such review serves the same purpose, and based on that determination, notify the submitter that no RAC review will take place, no NIH approval is necessary, and the experiment may proceed upon approval from the other Federal Agency."

Dated: November 12, 1985.

James B. Wyngaarden, M.D.,

Director, National Institutes of Health.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE
WORKING GROUP ON GRAM POSITIVE BACTERIA
MINUTES OF MEETING[1]

DECEMBER 2, 1985

The Working Group on Gram Positive Bacteria of the Recombinant DNA Advisory Committee was convened at 9:00 a.m. on December 2, 1985, at the National Institutes of Health, Building 31A, Conference Room 4, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. David Friedman was the Chair. The following people were present for all or part of the meeting:

Working Group members:

Richard Baltz	Paul Lovett
Donald Clewell	Frank Macrina
Mitchell Cohen	Peter Pattee
Donald Dean	Michael Thomashow
George Fox	Curtis Thorne
David Friedman	Elizabeth Milewski
Jack London	(Executive Secretary)

A working group roster is attached (Attachment I).

Other National Institutes of Health staff:

Susan Gottesman, NCI

Others:

Greg Pearson, Blue Sheet
Jane Rissler, Environmental Protection Agency
Mark Segal, Environmental Protection Agency

[1]The working group is advisory to the RAC, and its recommendations should not be considered as final or accepted.

40/5

Dr. Friedman called the Working Group on Gram Positive Bacteria to order at 9:00 a.m. on December 2, 1985. He said the purpose of the meeting was to evaluate a proposal (Attachment II) submitted by Drs. Richard Novick and Joan Polak of the Public Health Research Institute of the City of New York, Inc., New York, New York, to establish a comprehensive listing of gram-positive Eubacteriales under Section III-D-4 of the National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules.

Section III-D-4 states that certain recombinant DNA molecules are exempt from the NIH Guidelines. These exempt molecules "consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent." Organisms have been exempted under Section III-D-4 based on data documenting exchange of DNA between the organisms. Appendix A of the NIH Guidelines lists these exempt organisms.

Dr. Friedman called the attention of the working group to the philosophical basis used to construct Appendix A (Attachment III). In evaluating a pair of organisms for inclusion on a list of Appendix A, an estimate is made of the probability that the combination of genes might have occurred naturally. If the combination is not a "novel" one, there should be no special hazard in creating such an organism by recombinant DNA technology; and the combination of organisms can then be exempted from the NIH Guidelines. The natural transfer of genes between bacteria occurs by transduction, transformation, or conjugation. In 1978, in constructing Appendix A, four categories of mechanisms of conjugal genetic exchange were considered: (1) Organisms which exchange chromosomal genetic information which is stably integrated into the host chromosome. This most stringent criterion for exchange requires significant homology between the combining segments. (2) Organisms which exchange chromosomal information that is not necessarily integrated into the chromosome of the recipient. This sort of exchange can occur in the absence of extensive DNA homology between the organisms and requires only that the plasmid and its chromosomal genes be maintained in the recipient organism. (3) Organisms which show evidence of a plausible mechanism for exchange. In this case, the plasmid itself has been shown to move from organism to organism. It has been shown to pick up chromosomal genes but the transfer of these chromosomal genes in interspecies matings has not necessarily been demonstrated. (4) Organisms which can receive or donate broad host range plasmids. The assumption is that chromosomal genes may be picked up by these plasmids and transfer of chromosomal genes to other species will occur.

In 1978, the NIH Director decided the data supporting criteria 3 and 4 were not compelling enough to be used as a basis for exempting organisms from the NIH Guidelines.

Dr. Friedman said genetic material may be exchanged in one direction or in both directions, and organisms can be placed in a list in Appendix A on the basis of one-way exchange of genetic information.

Dr. Friedman said currently most experiments with gram-positive organisms not covered by Appendix A are covered by Section III-C. This section requires investigators to register with the local Institutional Biosafety Committee (IBC) at initiation of the experiments. The experiments can then proceed under Biosafety Level (BL) 1 containment conditions. Experiments involving Class 2 pathogens are covered by Section III-B, Experiments Which Require IBC Approval Before Initiation of the Experiment.

Dr. Friedman suggested the working group begin by examining the proposal (Attachment II) submitted by Drs. Novick and Polak.

Dr. Baltz asked whether the working group could first discuss the relevance of the criteria used for adding organisms to the sublists of Appendix A.

Dr. Clewell noted that the NIH Guidelines have been constructed in accordance with knowledge of E. coli genetics. Dr. Dean questioned whether criteria based on the genetics of gram-negative bacteria are appropriately applied to gram-positive bacteria. Exchange of DNA in gram-positive bacteria does not follow the model observed in gram-negative bacteria. Gram-positive bacteria do not have the same type of conjugative plasmids observed in E. coli or the mechanisms of genetic exchange observed in gram-negative bacteria. He thought plasmid exchange alone, whether or not it mobilizes chromosomal exchange, should be adequate evidence for including organisms on the sublists of Appendix A.

Dr. Lovett said in gram-negative bacteria, F' episomes can be generated in the laboratory and introduced into bacteria that would not otherwise exchange DNA. Since F' episomes are not known in gram-positive bacteria and chromosomal gene transfer by this type of conjugation has not been described in these organisms, the same test cannot be applied. It is likely, however, that F' episomes exist in gram-positive bacteria.

Dr. Baltz supported Dr. Dean's argument; he felt evidence of plasmid transfer should suffice for including organisms on Appendix A.

Dr. Friedman said the working group can offer other criteria for exemption to the Director, NIH, but whatever recommendation the working group offers should be supported by strong scientific arguments.

Dr. Thomashow said the working group is to evaluate the probability that the combination of genes constructed in the laboratory using recombinant DNA techniques might occur naturally. The exchange of chromosomal markers must be addressed if organisms are to be included on an Appendix A sublist on the basis of "non-novelty" of the exchange. How transfer occurs is not an issue, and the working group need not be concerned with the mechanism.

Dr. Friedman suggested the working group not attempt at this meeting to rewrite the philosophical underpinnings of the NIH Guidelines; he thought it would be simpler for the working group to operate within the existing historical context.

Dr. Milewski suggested the working group first evaluate the proposal submitted by Drs. Novick and Polak. If the working group feels it is appropriate, the working group might proceed to develop a motion to modify the NIH Guidelines or to discuss the criteria for exemption under Section III-D-4.

Dr. Clewell suggested the working group for the moment accept the historical context and evaluate the proposal submitted by Drs. Novick and Polak.

Dr. Macrina commented that the working group may wish to consider the importance of tranductional, conjugational, transpositional, and transformational exchange in considering chromosomal gene exchange.

Dr. Friedman did not think it important to describe the mechanism of transfer; rather the working group should simply consider whether transfer of genetic material occurs.

Dr. Baltz asked whether organisms can be added to Appendix A sublists on the basis of DNA homology. He said most Streptomyces species are closely related. On the basis of 23s ribosomal RNA (rRNA) homology, some investigators suggest all Streptomyces species are probably one and the same species.

Dr. Macrina thought DNA homology alone would not be a good criterion on which to add organisms to Appendix A. The numbers can be misleading as can be seen by comparison of sequence data and DNA hybridization data. For example, some genes are 40 to 50% homologous at the sequence level but do not hybridize even under low stringency conditions. On the other hand, homology in certain genes under standard DNA/DNA hybridization methodology can be quite high between phylogenetically distant species.

Dr. Fox said molecular taxonomists would argue DNA hybridization numbers in the latter case are meaningless; the important numbers in determining relatedness are sequence data on 23s ribosomal DNA. The highly conserved nature of rRNA DNA makes rRNA sequence homology a useful tool for investigating the relationships of phylogenetically distant organisms but it ceases to be useful for investigating relatedness in closely related organisms. Closely related organisms can show very high degrees of rRNA hybridizibility but display very different characteristics. E. coli and Salmonella, for example, display different biological behavior but possess essentially identical rRNA sequences. Thus, rRNA sequence relatedness studies indicate whether organisms are related but not whether the organisms would be classified within a species or genus. For example, B. subtilis and the Mycoplasmata possess very similar rRNAs but they are not classified within the same genus. A good method of using rRNA sequence homology data for taxonomic classification has not yet been developed.

Ribosomal sequence homology is a good method for investigating relationships between genera, but it is not obvious how rRNA homology could be applied to species definitions.

Dr. Fox added that rRNA sequence homology of the Streptomyces is quite high; perhaps in the 90% range. For comparison, the rRNA sequence homology between the Mycoplasmata and B. subtilis is probably in the 80% range.

Dr. Baltz said he might independently attempt to develop a proposal to exempt experiments involving the Streptomyces from the NIH Guidelines under Section III-D-4. He said the Streptomyces have been exempted by the British Genetic Manipulation Advisory Group (GMAG) from their guidelines. He felt a proposal to create an Appendix A sublist for Streptomyces which addresses the issues would be accepted by the Recombinant DNA Advisory Committee (RAC) and the NIH. He said in addition to a high level of DNA homology, conjugation in Streptomyces was demonstrated in the 1950s, and a number of plasmids are known which mobilize chromosomal genes.

Dr. Friedman called the attention of the working group to the first set of organisms proposed as exchangers of genetic information by Drs. Novick and Polak (Attachment II): B. cereus and B. thuringiensis.

Dr. Clewell asked whether B. thuringiensis is a B. cereus strain which has acquired the capacity to produce the crystal protein toxin. Dr. Thorne said it is very difficult to distinguish B. cereus strains from B. thuringiensis strains lacking the crystal protein plasmid. B. cereus and B. thuringiensis share a high degree of DNA homology ($\geq$ 90%).

Dr. Thorne said investigators have successfully transferred chromosomal genes by transduction between some B. cereus and B. thuringiensis strains. However, among other B. thuringiensis and B. cereus strains, chromosomal gene transfer by transduction cannot be demonstrated. Why the transferred DNA does not integrate into the chromosome in some strains is not known.

Dr. Clewell asked whether B. cereus and B. thurgiensis exchange chromosomal genes through plasmid association.

Dr. Thorne said "conjugative-like" plasmids transfer in both directions between B. cereus and B. thuringiensis; chromosomal gene transfer by these "conjugative-like" plasmids has not yet been demonstrated. However, the B. thuringiensis crystal protein gene can be transferred by the "conjugation-like" mechanism; and there is evidence suggesting the crystal protein gene can be integrated into the chromosome.

Dr. Dean said most of the crystal protein genes of B. thuringiensis strains are carried on plasmids. Some crystal protein genes are chromosomally located. There is strong homology between the plasmid-borne and chromosomal crystal protein genes, and the gene can probably jump from the plasmid to the chromosome. Inverted repeat sequences surround the crystal protein genes and integration is assumed to occur by this mechanism. Exchange between B. thuringiensis, B.

mechanism has been demonstrated. "Conjugation-like" transfer is a more general mechanism than generalized transduction since generalized transduction is limited by a number of factors extraneous to homology.

Dr. Pattee asked whether the crystal protein genes are stably integrated since they are part of a transposon. Dr. Dean said it is assumed the crystal protein gene is stably integrated in the chromosomal location; it can be lost from the plasmid location.

Dr. Baltz asked whether chromosomal genes bound by insertion sequences involved in illegitimate recombination would be considered legitimate candidates for inclusion on Appendix A of the NIH Guidelines.

Dr. Clewell said conjugative transposons probably hitchhike with plasmids even though they can conjugate on their own at a low frequency; but the issue is whether information demonstrating the transfer of chromosomal genes has been published. He said evidence indicating genetic exchange between B. cereus and B. thuringiensis shows the type of exchange described in criterion 4 (Attachment III).

Dr. Friedman asked the working group to comment on the data cited by Drs. Novick and Polak for B. subtilis and B. amyloliquefaciens (Attachment II). Dr. Dean said B. subtilis and B. amyloliquefaciens have been shown to exchange chromosomal genes; they are included on Sublist B of Appendix A.

Dr. Friedman asked the working group to consider the data cited by Drs. Novick and Polak for B. subtilis and S. pneumoniae (Attachment II).

Dr. Lovett said the evidence for genetic exchange cited by Drs. Novick and Polak describes transfer of extrachromosomal drug resistance genes.

Dr. Macrina said a vast amount of information has accumulated in the past five to ten years which demonstrates widespread dissemination of drug resistance genes among Staphylococcus, Streptococcus, and other closely related pathogens. These genes are found on transposons, plasmids, and at chromosomal locations. A growing body of evidence suggests transfer of drug resistance genes should be considered a separate type of genetic exchange. These plasmids exchange intragenerically as well as intergenerically but are probably not "conjugative" per se. The mechanism of transfer of drug resistance genes should probably be called "conjugation-like." Drs. Clewell, Dean, and Macrina said they knew of no instances where drug resistance plasmids mobilize chromosomal DNA.

Dr. Macrina said the drug resistance plasmids should more properly be called multiple host-range plasmids to distinguish these types of plasmids from the plasmids found in gram-negative bacteria.

Dr. Pattee said some investigators have reported some gentamycin plasmids can transfer chromosomal DNA, but these results have not been reproducible.

Dr. Clewell said the reference citing exchange between B. subtilis and S. pneumoniae offered by Drs. Novick and Polak is an example of the type of exchange described under criterion 4 (Attachment III).

Dr. Friedman then called the attention of the working group to the group of organisms for which Drs. Novick and Polak had offered references indicating that Staphylococcus carnosus could donate DNA to S. intermedius, S. epidermidis, S. capitis, S. caseolyticus, S. warneri, S. haemolyticus, and S. xylosus (Attachment II).

Dr. Pattee said there is some evidence in the literature of bacteriophage transfer of antibiotic resistance genes between Staphylococcus aureus and S. epidermidis. Dr. Lovett said it is very common for bacteriophages of gram-positive bacteria to grow on one host and pick-up genes. The bacteriophage will not plaque on any other host, but it can transfer its genes.

The working group agreed the reference cited by Drs. Novick and Polak did not demonstrate DNA transfer; rather it demonstrated the ability of the bacteriophage phiSK311 to infect a number of organisms. The working group agreed sufficient information to determine whether genetic exchange occurs in this group of organisms was not available.

Dr. Friedman asked the working group to next consider the data cited by Drs. Novick and Polak for S. pneumoniae and B. subtilis (Attachment II). The working group agreed the citation offered by Drs. Novick and Polak indicate this type of transfer would be described under criterion 4 (Attachment III).

The working group said the evidence indicating exchange between S. faecalis and B. thuringiensis (Attachment II) would also be the type of exchange described under criterion 4 (Attachment III).

Dr. Friedman called the attention of the working group to Dr. Novick's contention that S. faecalis can donate DNA to S. pyogenes, S. agalactiae, S. sanguis, S. salivarius, S. cremoris, Lactobacillus casei, Staphylococci, B. subtilis and Group F Streptococci (Attachment II).

Dr. Macrina said Streptococcus lactis should be added to Dr. Novick's list for completeness; chromosomal exchange between S. lactis and the other listed organisms has not yet been demonstrated; however, plasmid exchange can be shown to occur.

Dr. Clewell said some of the organisms in this grouping are already included on Sublist F of Appendix B; chromosomal gene exchange has been demonstrated between Streptococcus sanguis, S. pneumoniae, S. faecalis, S. pyogenes, and S. mutans. Dr. Clewell said at this time there is no evidence of chromosomal gene exchange in S. agalactiae, Group F Streptococcus, S. salivarius, S. cremoris, and Lactobacillus casei although plasmid exchange of the type described in criterion 4 has been demonstrated.

Dr. Macrina said Sublist E which specifies one-way transfer of DNA from S. mutans into S. sanguis is superfluous and should be modified since Streptococcus mutans is included in Sublist F with S. sanguis.

Dr. Clewell said taxonomists have suggested S. faecalis should be renamed Enterococcus faecalis because by DNA hybridization and rRNA sequence homology studies, it appears to be more closely related to the Bacillus than to the Streptococcus. The working group may wish to note this proposed reclassification.

Dr. Clewell said taxonomists have recently reclassified Streptococcus mutans into five different species; the name S. mutans now refers to only one of the five species listed in the most recent edition of Bergey's Manual of Systematic Bacteriology.

Drs. Clewell and Pattee said the Staphylococci in the previous grouping of Dr. Novick's proposal (Attachment II) were originally classified as S. epidermidis but were recently speciated into the several species listed in Attachment II. Dr. Fox said rRNA sequence data indicates the Staphylococcus are a closely related group of organisms.

Dr. Baltz suggested organisms which are the same species by DNA homology or other criteria and yet have been speciated and classified separately might be treated in a special manner under the NIH Guidelines. THe working group agreed the name S. epidermidis would refer to S. intermedius, S. capitis, S. caseolytius, S. warneri, S. haemolyticus, and S. xylosus.

Dr. Macrina said the next two groups of bacteria cited by Drs. Novick and Polak as demonstrating genetic exchange are: (1) S. anginosus and S. agalactiae donating DNA to S. faecalis; and (2) S. faecalis and S. agalactiae donating DNA to S. agalactiae and S. faecalis (Attachment II). These references demonstrate interspecific transfer of extrachromosomal DNA among gram-positive bacteria. There is no evidence of chromosomal gene transfer.

Dr. Clewell said S. anginosus is a Group F species and probably the species used to represent the Group F Streptococci in tests of DNA transfer. He thought S. anginosus should be specifically named in any classification.

The working group agreed all of the references cited to demonstrate exchange between the bacterial groups listed by Drs. Novick and Polak demonstrated the plasmid transfer described under criterion 4 (Attachment III). Drs. Novick and Polak provide no references demonstrating chromosomal gene transfer.

Dr. Baltz asked whether investigators have looked extensively for chromosomal transfer in gram-positive organisms. Dr. Macrina said few investigators have searched for chromosomal gene transfer. He could, nonetheless, hypothesize a mechanism in gram-positive bacteria by which a transposon could integrate in the chromosome, become a transposon prime, hop onto a plasmid to create a R' plasmid, and transfer chromosomal genes to other bacteria.

Dr. Clewell said his laboratory performed some studies several years ago on the mobilization by conjugative plasmids of chromosomal genes in S. faecalis. Transfer was seen at the frequency of 10^{-7} per donor. This study is the only published demonstration of mobilization of chromosomal markers in gram-positive bacteria.

Dr. Pattee said his laboratory had investigated S. faecalis/S. aureus pairs and had only observed plasmid transfer.

Dr. Dean said some data on the chromosomal transfer of chromosomally-located streptomycin resistance genes between B. cereus and B. thuringiensis exist but these data have not been published.

Dr. Friedman introduced to the working group Dr. Susan Gottesman of the NIH. He asked Dr. Gottesman, a member of the RAC, to describe the philosophical basis of Appendix A of the NIH Guidelines.

Dr. Gottesman said under Section III-D-4 organisms which exchange DNA by known physiological processes have been exempted from the NIH Guidelines. The argument supporting this exemption is the following: if exchange of chromosomal genes can be shown to occur under natural conditions, no novel combinations would arise in the laboratory through use of recombinant DNA techniques.

Dr. Gottesman said in 1978, RAC accepted evidence of chromosomal exchange between organisms as an indication that no "novel" organism would be created through laboratory combination of the genes of these organisms; RAC also accepted data demonstrating plasmid mobilization of chromosomal genes as an indication no "novel" organisms would be created. Later, information in gram-negative bacteria demonstrating that chromosomal gene exchange occurred at some frequency with plasmid exchange accumulated. Eventually, for gram-negative bacteria RAC accepted that plasmid exchange could result in transfer of chromosomal genes.

Dr. Cohen asked whether the working group would have to demonstrate that exchanges occur in nature to support their recommendation. Was the effect of different ecological niches on the transfer frequency considered when the gram-negative bacteria were evaluated for inclusion in Appendix A? Dr. Gottesman replied that if exchange could be shown in the laboratory, RAC has accepted the argument that given enough time and space such genetic exchange could occur in nature. She said RAC did not consider the effect of different ecological niches on the probability of exchange.

Dr. Baltz asked whether RAC considered the effect of site-directed changes on organisms exempted from the NIH Guidelines. Site-directed mutagenesis speeds up the process of evolution and might generate an organism which had not arisen in nature. Dr. Gottesman said RAC has chosen to ignore the effect of site-directed mutagenesis on evolution. However, any testing in the environment of a modified organism must be reviewed by RAC and approved by NIH and the IBC even if laboratory manipulations involving the modified organism are exempt from the NIH Guidelines.

Dr. Thomashow asked whether the working group could argue that certain organisms ought to be exempt on the basis of sequence homology. Dr. Gottesman replied that an argument based on a high degree of homology, e.g., 90% homology in a randomly selected gene and not a highly conserved gene, would be acceptable.

Dr. Dean thought a high degree of homology would argue that no new organism would be created by use of recombinant DNA techniques since any change would be within the evolutionary range of the bacteria.

Dr. Baltz asked whether organisms could be added to Appendix A if exchange could be shown between organisms by the mechanism of phage transduction. Dr. Gottesman replied such an argument would have to be supported by knowledge about the transduction mechanism. It does not matter what the mechanism of exchange of chromosomal material is as long as exchange can be shown to occur.

Dr. Baltz said chromosomal gene transfer by plasmids has been shown in Streptomyces but not yet in Streptococcus or Staphylococcus. Since Streptomyces are gram-positive bacteria can these observations be extrapolated to Streptococcus and Staphylococcus?

Dr. Gottesman said it would be difficult to extrapolate from the Streptomyces to the Streptococcus and Staphylococcus if the plasmids and the mechanisms are different in the Streptomyces; arguments advanced in support of a recommendation should be supported scientifically.

Dr. Friedman asked whether the pathogenicity of certain Streptococcus and Staphylococcus will affect RAC's deliberations.

Dr. Gottesman said the fact these organisms are pathogens will reflect on any recommendation since the question is whether a better pathogen can be constructed by recombinant DNA methods than would occur in nature. Another issue is whether records of a pathogen's construction and use should be kept. She reminded the group that most experiments involving gram-positive bacteria can currently be performed at BL1 containment with IBC registration or notification.

Dr. Clewell asked how RAC views sublists of Appendix A which include human pathogens. Dr. Gottesman said the organisms included in sublists of Appendix A are exempt from the NIH Guidelines because they exchange genetic information. Organisms exempted because they are considered to be safe hosts are listed in Appendix C.

Dr. Gottesman said RAC's job is easier if working group recommendations are very clearly drawn. She recommended a proposal be written so that even if RAC does not accept the whole recommendation, it could accept some part of the recommendation. The working group should not attempt to squeeze the science to fit the recommendation but should craft the recommendation to fit the science. She suggested the working group outline the strongest scientific arguments supporting their recommendation(s).

Dr. Gottesman said the working group could construct a recommendation on one of several methods of exempting experiments from the NIH Guidelines: (1) The working group could attempt to add organisms to Appendix A on the basis of plasmid exchange using the broadest definition of exchange. (2) Another approach would be to attempt to exempt organisms under Appendix C. Experiments involving the cloning of genes in E. coli K-12, Saccharomyces cerevisiae, asporogenic Bacillus subtilis host-vector systems or in tissue culture, are exempt under Appendix C of the NIH Guidelines based on the argument that these organisms would not present a hazard. A generic statement of this type would probably not work, however, for groupings including pathogens. (3) A third approach would be to exempt experiments involving the DNA of the broad host-range plasmids which exchange freely within the gram-positive bacteria when propagated and maintained in those bacteria.

Dr. Cohen reiterated that all experiments involving organisms being considered by the working group are currently permitted under the NIH Guidelines. He questioned whether any advantage would be gained in modifying the NIH Guidelines regarding these organisms.

Dr. Dean said the major impact would be on large-scale work with gram-positive bacteria since large-scale procedures must be reviewed and approved by the IBC prior to initiation of the experiments. Dr. Baltz said a cost factor must also be considered when procedures involving these organisms are scaled-up.

Dr. Dean said it might be useful at this time to recommend the third option of exempting experiments involving those plasmid-borne genes known to be exchanged among gram-positive bacteria. Procedures for certain types of experiments would be simplified, e.g., research with the B. thuringiensis plasmid-borne crystal protein or with drug resistance genes. An established list of gram-positive organisms which exchange plasmids might make it easier to exempt these organisms as a group as evidence of chromosomal exchange is generated.

Dr. Lovett questioned whether a great deal would be gained by creating such an exempt list of broad host-range plasmids when those plasmids are propagated and maintained in gram-positive bacteria. B. thuringiensis and B. subtilis would be on this list but might also be exempted from the NIH Guidelines on the basis of a high level of DNA homology. B. thuringiensis is not a hazard for humans, and this argument might support exempting this organism under Appendix C.

Dr. Gottesman said exempting an organism such as B. thuringiensis which is an insect pathogen would entail consideration of host range. Issues that would have to be considered include: what factors control host range and could modification of the host range adversely affect beneficial insects?

Dr. Dean thought investigators encounter problems when they scale-up manipulations. He thought industrial IBCs tend to be very conservative; language exempting extrachromosomal DNA from the NIH Guidelines would simplify matters for individuals working with extrachromosomal DNA from gram-positive bacteria.

He suggested it would also be very helpful if shuttle vectors composed of E. coli and S. cerevisiae DNA could be exempt when used with these organisms and broad host-range plasmids. Dr. Thomashow agreed.

Dr. Lovett said some data demonstrate that one of the broad host-range gram-positive plasmids replicates in E. coli and S. cereviseae.

Dr. Gottesman thought a proposal including shuttle vectors composed of E. coli and S. cerevisiae DNA would be acceptable. She noted that earlier versions of the NIH Guidelines permitted DNA cloned in E. coli to be "returned to host of origin" with the plasmid vector. This language permitted the investigator to clone DNA in E. coli host-vector systems and return the vector containing the cloned DNA to the original DNA source organism without considering the effect of the vector DNA on the host of origin. She suggested language concerning the use of shuttle vectors could be added to the third option.

Dr. Macrina said he preferred the first option although he did not know if sufficient information exists to support this position. He felt the third option could for the most part already be found in the NIH Guidelines.

Dr. Lovett supported the first option; he felt extensive plasmid exchange occurs in the gram-positive bacteria although there are no mechanisms currently known which permit chromosomal gene exchange by these plasmids.

Dr. Cohen felt not enough information is available to support the first option. Dr. Thomashow did not think the working group should offer a recommendation which cannot be supported by data.

Dr. Clewell said the third option does have merit; it would simplify the situation for large-scale procedures and would clarify the language of the NIH Guidelines concerning use of these types of broad host-range plasmids. He did not think sufficient evidence of chromosomal exchange exists to support the first option and noted that significant human pathogens are in this group of bacteria.

Dr. Thorne said philosophically he supported the first option but did not feel sufficient evidence existed to support a recommendation exempting organisms from the NIH Guidelines on the basis of chromosomal gene exchange. He, therefore, recommended the working group develop the third option.

Dr. Cohen asked how Section III-A of the NIH Guidelines impacts Appendix A. Would the presence of drug resistance genes on broad host-range plasmids present a problem for a recommendation exempting these plasmids used in gram-positive bacteria?

Dr. Gottesman replied that Section III-A, Experiments Which Require Specific RAC Review and NIH and IBC Approval Before Initiation of the Experiment, overrides other sections of the NIH Guidelines; experiments which are covered by both Section III-A and III-D would not be exempt. Section III-A-3 of the NIH

Guidelines addresses the deliberate introduction of drug resistance traits to organisms which would not ordinarily naturally acquire it in any other way if the acquisition could compromise the use of the drug to control disease agents in human or veterinary medicine or agriculture.

Dr. Macrina did not think organisms created in the laboratory by the introduction of antibiotic resistance genes would be worse than those found in nature. He thought the potential for assortment of colonization and virulence factors exists in nature.

Dr. Pattee noted that chromosomally located pathogenicity determinants do not appear to be transferring from organism to organism in nature.

Dr. Dean asked whether any additional issues are raised by cloning antibiotic resistance genes on shuttle vectors. Dr. Friedman said Section III-A-3 would address that issue.

Dr. Gottesman suggested the third option could be constructed under Section III-D-5 of the NIH Guidelines; the language could appear as Appendix C-V, Extrachromosomal Elements of Gram-Positive Organisms.

Over lunch a subworking group developed the following proposed language:

> "Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below including shuttle vectors with E. coli replicons that are propagated and maintained in organisms listed below are exempt from these Guidelines."

Dr. Thomashow said two rationales support this recommendation: (1) data demonstrate extensive intergeneric transfer of broad host-range plasmids; and (2) data demonstrate intergeneric transfer of nonconjugative plasmids between the organisms on the list.

Dr. Baltz asked whether use of shuttle vectors constructed from S. cerevisiae replicons would be acceptable and could be added to the language. Dr. Dean agreed with this position. He felt S. cerevisiae DNA might be part of useful shuttle vectors. He suggested DNA from organisms described in Appendix C, i.e., E. coli K-12, S. cerevisiae, and asporogenic B. subtilis be exempted when part of shuttle vectors.

Dr. Cohen said an argument for permitting use of shuttle vector DNA in gram-positive organisms is that these shuttle vectors are well-known safe vectors which have been extensively studied in E. coli and/or S. cerevisiae, or their native hosts. Dr. Thorne said these vectors do not have any toxic or biologically hazardous characteristics.

Dr. Thomashow questioned whether the word "replicon," a rather undefined term, should be part of the language of this recommendation. Dr. Baltz suggested

the language simply state that shuttle vectors "constructed from vectors described in Appendix C" are permissible. Dr. Dean felt the language should be as general as possible and agreed with Dr. Baltz's suggestion. The working group agreed.

Dr. Cohen asked whether the DNA of the phage vectors described in Appendix C might be included under the exemption. The working group thought this would be reasonable in order to permit use of cosmid vectors. They noted the term "extrachromosomal elements" includes phage vectors.

Dr. Macrina asked how a conjugative transposon that exists only on the chromosome would be considered. Is it indigenous chromosomal material; if it can move to other organisms, would it be considered extrachromosomal?

Dr. Clewell said conjugative transposons in gram-positive bacteria do not appear to transfer chromosomal genes.

Dr. Cohen asked how phage integrated into the chromosome would be handled under this proposal. Dr. Lovett offered the example of a defective temperate Bacillus subtilis bacteriophage which can replicate extrachromosomally when cloned in a recombinant minus (rec^-) bacteria. Dr. Macrina said clostridial plasmid probes hybridize to the DNA of plasmid-less Clostridia strains. These integrated plasmids apparently do not come out of the chromosome. He hypothesized integrated plasmids may occur widely in the understudied gram-positive organisms.

The working group decided integrated plasmids, transposons, or phages which do not come out of the chromosome would not be considered extrachromosomal elements under this proposal.

Dr. Thomashow asked whether there is evidence of transfer of non-mobilizable plasmids by "conjugative-like" plasmids in gram-positive bacteria. Dr. Clewell said non-mobilizable plasmids are transferred by "conjugative-like" plasmids but the mechanism by which this occurs is not known. Dr. Macrina hypothesized some of the non-mobilizable plasmids may be transferred by mob mechanisms; others may be transferred by co-integration into "conjugative-like" plasmids.

Dr. Dean said there is extensive DNA homology between the plasmids and the transposons covered by the working group proposal. Dr. Lovett agreed; he offered the example of an apparently identical plasmid independently isolated from three different sources: the plasmid pAM-alpha-1 from S. faecalis; the plasmid pBC16 from Bacillus cereus; and the plasmid PUB110 from S. aureus. The high degree of homology observed in these plasmids suggests recent lateral transfer of the plasmid between these organisms. Dr. Macrina offered the example of two transposons which appear to be identical although isolated from different sources: the transposon TN917 isolated from an enteric Streptococcus and the transposon TN551 isolated from S. aureus.

The working group then began to construct the list of organisms which exchange broad host-range plasmids. They accepted most of the organisms listed by Drs. Novick and Polak in their proposal.

Dr. Dean suggested Clostridium acetobulyticum and S. lactis be added to the list on the basis of data indicating plasmid exchange.

Dr. Macrina suggested S. mutans which had inadvertently been omitted from Appendix A be added to Sublist F of Appendix A and to any list compiled by the working group. He said evidence would support the addition of S. thermophylus, S. milleri, S. durans, S. mitior, and S. ferus to the list. He also suggested Pediococcus pentasaceus, P. acidilactici and P. damnosus be added to the list.

Dr. Clewell suggested S. sobrinus be added. Dr. Dean suggested B. brevis, B. anthracis, and B. megaterium be added. He said the organisms from sublist B of Appendix A which should be added are B. natto, B. aterrimus, B. niger, and B. globigii. He pointed out that both B. natto and B. globigii are classified as B. subtilis.

Dr. Dean called attention to the letter from Ms. Alice Caddow of Genencor (Attachment IV). Ms. Caddow had suggested Bacillus amylosaccharitícus be added to any list of organisms exchanging DNA with the Bacilli. Dr. Dean supported the addition of B. amylosaccharitícus to the list being developed by the working group.

Dr. London said only one-way transfer of DNA from the Streptococci into Lactobacillus casei has been demonstrated. The working group discussed whether evidence of one-way transfer would be adequate for addition of the organism to the list. They agreed an argument could be made to RAC to include Lactobacillus casei on the list on the basis of one-way transfer of genetic information.

The working group agreed proposed Appendix C-V, Extrachromosomal Elements of Gram Positive Organisms, would read as follows:

> "Recombinant DNA molecules derived entirely from extrachromosomal elements of the organisms listed below (including shuttle vectors constructed from vectors described in Appendix C) and propagated and maintained in organisms listed below are exempt from these Guidelines.
>
> Bacillus subtilis
> Bacillus pumilus
> Bacillus licheniformis
> Bacillus thuringiensis
> Bacillus cereus
> Bacillus amyloliquefaciens
> Bacillus brevis
> Bacillus natto
> Bacillus niger
> Bacillus aterrimus
> Bacillus amylosaccharitícus
> Bacillus anthracis

Bacillus globigii
Bacillus megaterium
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus carnosus
Clostridium acetobutylicum
Pediococcus damnosus
Pediococcus pentosaceus
Pediococcus acidilactici
Listeria grayi
Listeria murrayi
Listeria monocytogenes
Streptococcus pyogenes
Streptococcus agalactiae
Streptococcus sanguis
Streptococcus salivarious
Streptococcus cremoris
Streptococcus pneumoniae
Streptococcus avium
Streptococcus faecalis
Streptococcus anginosus
Streptococcus sobrinus
Streptococcus lactis
Streptococcus mutans
Streptococcus equisimilis
Streptococcus thermophylus
Streptococcus milleri
Streptococcus durans
Streptococcus mitior
Streptococcus ferus

Dr. Fox said all the organisms on the list generated by the working group represent a coherent evolutionary group with the exception of Clostridia acetobutylicum.

Dr. Thorne suggested the first paragraph of Appendix C-IV should be modified. That paragraph currently reads as follows:

> "Any asporogenic Bacillus subtilis strain which does not revert to a sporeformer with a frequency greater than 10^{-7} can be used for cloning DNA with the exception of those experiments listed below. Indigenous Bacillus plasmids and phages whose host-range does not include Bacillus cereus or Bacillus anthracis may be used as vectors."

Dr. Thorne suggested the second sentence of this paragraph be deleted. He argued that a great deal of evidence demonstrates plasmid exchange between B. anthracis, B. cereus and other gram-positive organisms. Exchange of plasmids between these organisms would occur in nature.

Dr. Dean said the rationale for restricting plasmids whose host-range includes B. cereus was that some B. cereus strains produce an enterotoxic food toxin. On the basis of exceptional B. cereus strains, all plasmids which can be introduced into B. cereus were eliminated from use under Appendix C-IV.

The working group supported this proposal and agreed to offer it as a recommendation to RAC.

Dr. Friedman adjourned the meeting of the Working Group on Gram Positive Bacteria at 3:05 p.m. on December 2, 1985.

[Executive Secretary's Note: The proposal offered by Drs. Novick and Polak cite references which support the working group recommendation. The working group in addition has compiled a reference list supporting its recommendation. That reference list is appended to these minutes as Attachment V.]

Respectfully submitted,

Elizabeth Milewski

Elizabeth A. Milewski, Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

1/27/86
Date

David I. Friedman

David I. Friedman, Ph.D.
Chair
Working Group on Viruses

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TO: Recombinant Advisory Committee

FROM: J. Polak and R. Novick

RE: Exchanger list for gram positive bacteria

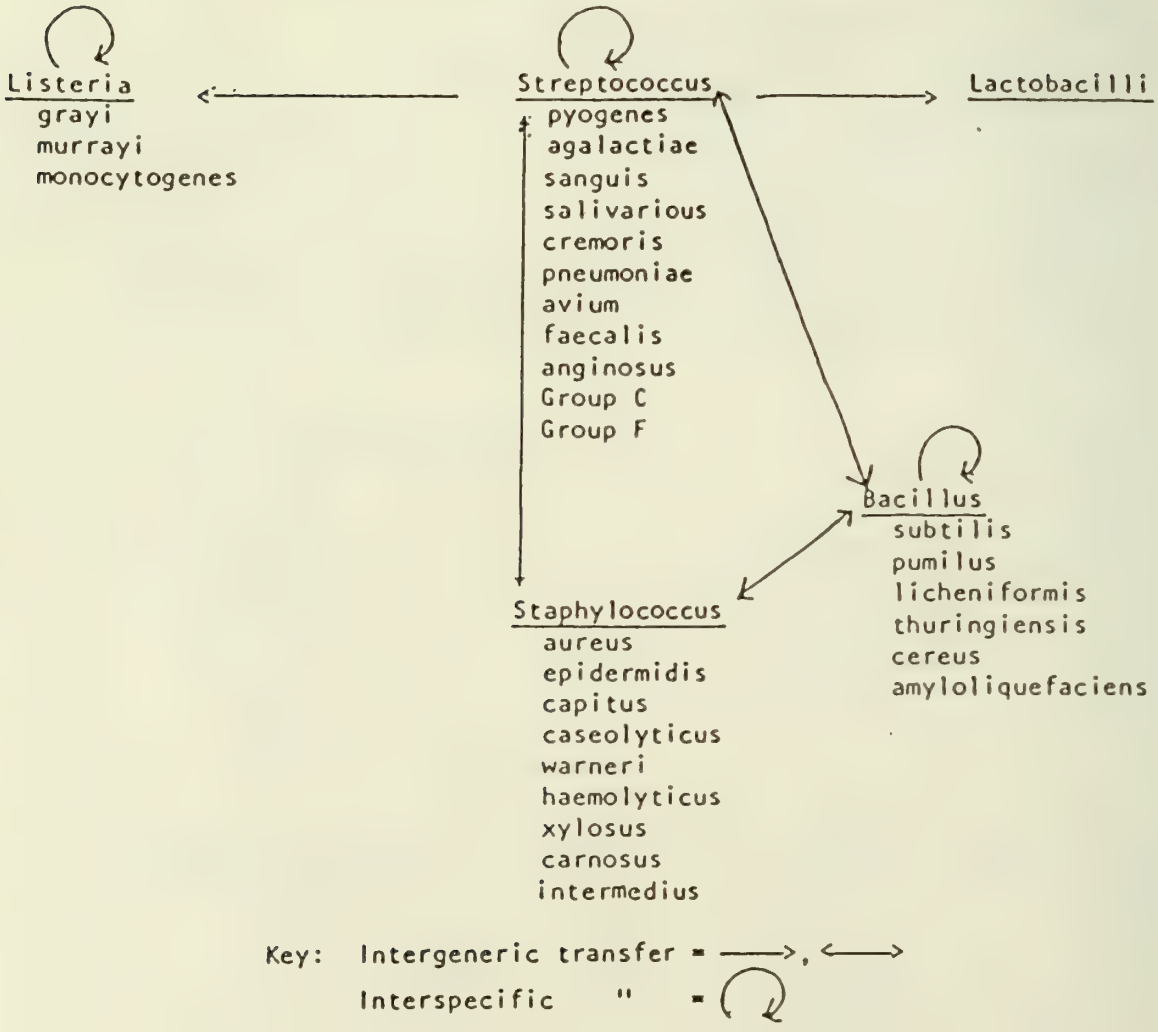
Att: Dr. William Gartland

On the basis of the attached compilation reviewing the range of extra-chromosomal transfer among gram positive bacteria (see Tables A and B) we should like to propose the establishment of a comprehensive exchanger list for the gram positive Eubacteriales, including the following genera and species:

Bacillus	cereus
	subtilis
	thuringiensis
	amyloliquefaciens
	pumilus
	stearothermophilus
	sphaericus
	lichenformis
Streptococcus	pneumoniae
	faecalis
	pyogenes
	agalactiae
	sanguis
	anginosus
	salivarius
	cremoris
	avium
Other Groups	Group F
	Group C
	Lactobacillus casei
Staphylococcus	aureus
	epidermidis
	carnosus
	xylosus
	intermedius
	capitis
	warneri
	haemolyticus
	intermedius
Listeria	monocytogenes
	grayi
	murrayi

For the above listed organisms the following exchange pattern has been demonstrated by use of natural means of genetic transfer (transduction, conjugation, competent cell transformation, mixed culture mechanism unspecified -. See Table A):

Summary of transfer pattern based on Table A



Key: Intergeneric transfer = ⟶, ⟷

Interspecific " = ↻

Parallel exchange patterns can also be inferred on the basis of natural carriage of highly related or indistinguishable plasmids (see Table B) as shown below:

Transfer pattern based on Table B

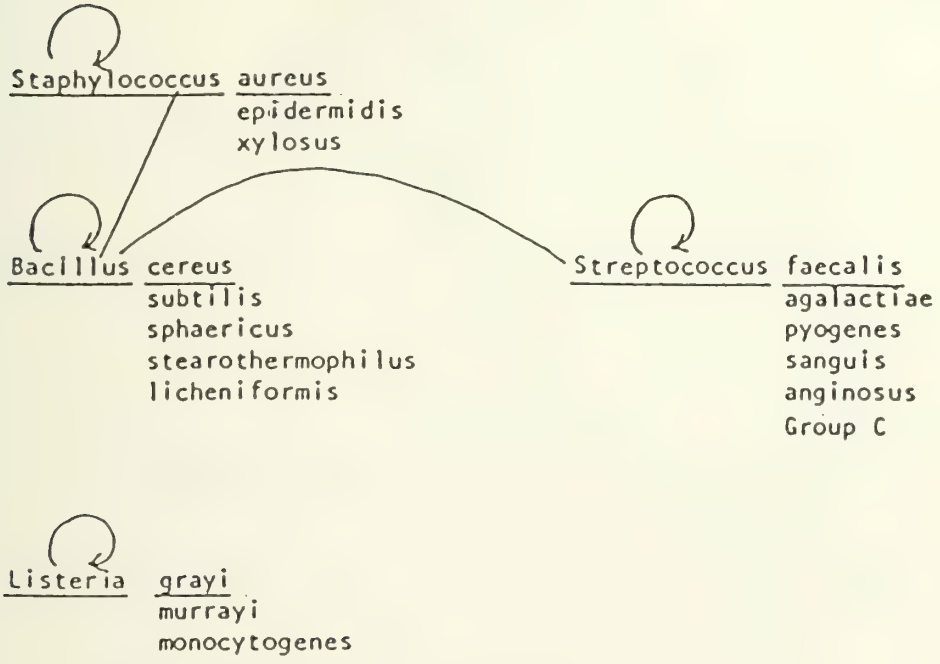


TABLE A

Interspecific transfer of extra-chromosomal DNAs among gram-positive bacteria

Donor	Recipient	Extra-chromosomal DNA transferred	Mode of transfer	Reference
B. cereus	*B. thuringiensis*	plasmids; pBC16(Tc^r); pC194(Cm^r)	transduction	(1)
B. thuringiensis	*B. cereus*	plasmids; pBC16(Tc^r); pC194(Cm^r)		
B. subtilis	*B. amyloliquefaciens*	plasmid pUB110 derivatives (Km^r)	transduction	(2)
B. subtilis	*S. pneumoniae*	plasmids; pMP2(Km^r)	competent cell transformation	(3)
S. pneumoniae	*B. subtilis*	plasmids; pLS1(Tc^r) pHV158(Tc^r)		
S. carnosus	*S. intermedius* *S. epidermidis* *S. capitis* *S. caseolyticus* *S. warneri* *S. haemolyticus* *S. xylosus*	bacteriophage ϕSK311	transduction	(4)
S. pneumoniae	*B. subtilis*	plasmid pLS80 derivatives (Sul^r)	competent cell transformation	(5)
S. faecalis	*B. thuringiensis*	plasmid pAMβ1 (MLS^r)	conjugation	(6)
B. thuringiensis	*B. cereus*	δ-endotoxin plasmid	likely conjugation	(7)
S. faecalis	*S. pyogenes* *S. agalactiae* Group F *S. faecalis* *S. sanguis* *S. salivarius* *S. cremoris* *Lactobacillus casei*	plasmid pAMβ1 (MLS^r)	conjugation	(8)
Group F Streptococcus	Staphylococci *B. subtilis*			

Interspecific transfer of extra-chromosomal DNAs among gram-positive bacteria

Donor	Recipient	Extra-chromosomal DNA transferred	Mode of transfer	Reference
S. anginosus S. agalactiae	S. faecalis	plasmid: pIP659 (MLS^r) pI501 (MLS^r)	conjugation	(9)
S. faecalis S. agalactiae	S. agalactiae S. faecalis	pRI402 (Em^r)	conjugation	(10)
S. aureus	S. pyogenes S. agalactiae S. faecalis	pRI405 (MLS^r)	conjugation	(11)
S. faecalis	S. aureus	pAMβ1 (MLS^r)		
Group F Streptococcus	S. avium Lactobacillus casei	pAMβ1 (MLS^r)	conjugation	(12)
S. faecalis	S. pyogenes S. agalactiae	plasmids (MLS^r)	conjugation	(13)
S. agalactiae	S. faecalis S. sanguis Group F			
Group F	S. faecalis			
S. sanguis	S. faecalis			
S. aureus	S. epidermidis	Gentamicin-resistant plasmids	Unknown (transfer observed in mixed culture & on human skin)	(14)
S. epidermidis	S. aureus			
B. subtilis	S. aureus	Plasmids: pC194 (Cm^r)	competent cell transformation	(15)
S. epidermidis		pTE109 (Tc^r) pCE109 (Cm^r)		

TABLE A (Cont'd)

Interspecific transfer of extra-chromosomal DNAs among gram-positive bacteria

Donor	Recipient	Extra-chromosomal DNA transferred	Mode of transfer	Reference
B. subtilis	*B. pumilus* *B. licheniformus*	Plasmid pUB110 (Km^r)	transduction	(16)
Streptococcus: Group C	*S. faecalis* *S. agalactiae*	Plasmids: pIP646 (MLS^r)	conjugation	(17)
S. anginosus	*S. faecalis*	pI920 (MLS^r)		
S. agalactiae	Listeria: *L. monocytogenes* *L. grayi* *L. murrayi*	Plasmid pI501	conjugation	(18)
S. aureus	*B. subtilis*	pC194 (Cm^r) pT127 (Tc^r)	competent cell transformation	(19)
S. faecalis	*B. subtilis*	pAMα1	competent cell transformation	(20)

TABLE 8

Natural transfer as indicated by indistinguishable or highly related plasmids or transposons found amoung diverse gram + species.

Extra-chromosomal DNAs	Occurs Naturally In	Reference
Represented by Plasmid pBC16	Bacillus cereus	21, 22
	Bacillus subtilis	22
	Bacillus licheniformis	22
	Bacillus sphaerius	22
	Bacillus stearothermophilus	23, 22, 24, 25
	Staphylococcus aureus	22
	Streptococcus faecalis	20
Represented by Arsenate operon from plasmid pI258	Staphylococcus xylosus	26
	Staphylococcus aureus	27
Represented by plasmid pIP646	Streptococcus anginosus	32
	Streptococcus Group C	32
Represented by erythromycin resistant transposon Tn551 from plasmid pI258	Staphylococcus aureus	33
	Streptococcus sanguis	33
	Streptococcus pneumoniae	33
	Streptococcus pyogenes	33, 34
	Streptococcus faecalis	34
	Streptococcus agalactiae	34
Represented by plasmid pIP501	Streptococcus agalactiae	35
	Streptococcus faecalis	35
Represented by Plasmid pRYC16	Listeria murrayi	18
	Listeria grayi	18
	Listeria monocytogenes	18
Represented by plasmid pT181	Staphylococcus aureus	36
	Staphylococcus epidermidis	36

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ATTACHMENT III OF THE MINUTES OF THE DECEMBER 2, 1985, MEETING OF THE RAC WORKING GROUP ON GRAM POSITIVE BACTERIA IS PAGE 60099 TO PAGE 60101 OF THE DECEMBER 22, 1978, FEDERAL REGISTER WHICH CAN BE FOUND IN VOLUME 4 OF RECOMBINANT DNA RESEARCH.

GENENCOR

Genencor, Inc. 180 Kimball Way South San Francisco, CA 94080 Telephone 415-588-3475

November 15, 1985

Dr. Elizabeth Milewski
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Committee Working Group
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Dear Dr. Milewski

As we discussed today, Genencor has identified several species of Bacillus which are indicated in the literature to exchange genetic material and which were not listed in the memo from J. Polak and R. Novick to the Recombinant DNA Advisory Committee. These organisms are listed below and the literature references are included for your use.

Bacillus subtilis
var. aterrimus
var. natto (or B. natto)
var. niger (or B. niger)

Bacillus polymyxa ATCC 842

— J. Marmur, et al J. Bacteriol(1963) 85,461-467.

[although there is another literature report that B. polymyxa NRRL B367 does not transform B. subtilis(Dubnau, et al, Genetics (1965) 54:491-498)]

Bacillus amylosacchariticus or B. subtilis var. amylosacchariticus — Yoneda, Y., et al J. Bacteriol(1974) 120: 1144-1150.

In addition, there is a report in the literature that alkalophilic bacilli exchange genetic material with B. subtilis (Takinishi, H.,et al, FEBS Letters(1983)154: 201-204).

We respectfully request that the Working Group consider adding the bacilli listed above to the exchanger list.

If you have any questions on the enclosed material, please do not hesistate to contact me.

Sincerely,

Alice J. Caddow

Alice J. Caddow
Manager of Regulatory Affairs

enclosures

INCLUDED IN ATTACHMENT IV OF THE MINUTES OF THE DECEMBER 2, 1985, MEETING OF THE RAC WORKING GROUP ON GRAM POSITIVE BACTERIA ARE THE ARTICLES:

"INTERSPECIFIC TRANSFORMATION IN BACILLUS." J. MARMUR, E. SEAMAN AND J. LEVINE 1963. JOURNAL OF BACTERIOLOGY, VOLUME 85, PAGES 461-467.

"GENE CONSERVATION IN BACILLUS SPECIES, I. CONSERVED GENETIC AND NUCLEIC ACID BASE SEQUENCE HOMOLOGIES." D. DUBNAU, I. SMITH, P. MORELL AND J. MARMUR. 1965 GENETICS, VOLUME 54, PAGES 491-498

"TRANSFORMATION OF BACILLUS SUBTILIS IN ALPHA AMYLASE PRODUCTIVITY BY DEOXYRIBONUCLEIC ACID FROM B. SUBTILIS VAR. AMYLOSACCHARITICUS." Y. YONEDA, K. YAMANE, K. YAMAGUCHI, Y. NAGATA AND B. MARUO. 1974 JOURNAL OF BACTERIOLOGY, VOLUME 120, PAGES 1144-1150

"DNA FROM ALKALOPHILIC BACILLUS CAN TRANSFORM B. SUBTILIS TO ALKALOPHILY." H. TAKINISHI, T. SEKIGUCHI, N. KOYAMA, K. SHISHIDO AND Y. NOSOH. 1983 FEBS LETTERS, VOLUME 154, PAGES 201-204.

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41/43

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DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

RECOMBINANT DNA ADVISORY COMMITTEE
WORKING GROUP ON HUMAN GENE THERAPY

MINUTES OF MEETING[1]

DECEMBER 16, 1985

The Working Group on Human Gene Therapy of the Recombinant DNA Advisory Committee was convened at 9:00 a.m. on December 16, 1985, at the National Institutes of Health, Building 31, Conference Room 6, 9000 Rockville Pike, Bethesda, Maryland 20892. Dr. LeRoy Walters was Chair. The following were present for all or part of the meeting:

Working Group members:

W. French Anderson
Judith Areen
Alexander Capron
James Childress
Samuel Gorovitz
Susan Gottesman
Clifford Grobstein
Maurice Mahoney
Arno Motulsky
Robert Rich
Harold Varmus
LeRoy Walters
Anne Witherby
William Gartland
(Executive Secretary)

A working group roster is attached (Attachment I).

Liaison representatives:

Bonnie Lee, Food and Drug Administration
Henry Miller, Food and Drug Administration

Ad hoc consultants:

Samuel Ackerman, Food and Drug Administration
George Scangos, Johns Hopkins University

Other National Institutes of Health staff:

Stanley Barban, NIAID
Prabhakara Choudary, NINCHS
Rachel Levinson, OD
Elizabeth Milewski, NIAID

Others:

Philip Chao, Food and Drug Administration
Jeff Christy, Blue Sheet
Robert Cook-Deegan, Office of Technology Assessment

[1]The working group is advisory to the RAC, and its recommendations should not be considered as final or accepted.

Dr. Walters convened the meeting of the Working Group on Human Gene Therapy at 9:15 a.m. on December 16, 1985. He said several topics would be covered at this meeting: (1) retroviral vectors; (2) the November 12, 1985, meeting of the RAC Working Group on Viruses; (3) transgenic animal studies; (4) the Food and Drug Administration (FDA) process for filing a "Notice of Claimed Investigational Exemption for a New Drug" (IND); and (5) other issues and announcements.

Retroviral Vectors

Dr. Anderson gave a presentation on the use of retroviral vectors to transfer genes in human gene therapy. He explained that retroviruses are animal viruses with a glycoprotein envelope and an RNA genome that replicate through a DNA intermediate. The DNA intermediate is stably integrated into cellular DNA; the integrated DNA is termed the provirus. Dr. Anderson said at this time many basic retroviral functions are not fully understood.

Dr. Anderson described the retrovirus life cycle (Attachment II). He said the virus recognizes sites on the cell membrane and adsorbs to these receptor sites. After penetration of the cell membrane, the single-stranded RNA genome is reverse transcribed in the cytoplasm to a double-stranded circular DNA molecule. The circular double-stranded molecule is transported into the nucleus and integrates into the host chromosome by a highly efficient ordered process. The integrated provirus serves as the template for synthesis of mRNAs encoding the gag, pol, and env functions as well as full-length transcripts of the viral genome.

Two viral long terminal repeats (LTRs) are situated at the junctions between viral and cellular genomes. Functional and nucleotide sequencing studies of LTRs derived from a number of different retrovirus isolates suggest that LTRs provide a number of functions essential to the retrovirus life cycle. These functions include provirus integration into host DNA, viral DNA synthesis, and transcription of the viral genome. The LTR on the 5' region of the provirus genome contains the functional transcriptional initiation site, while the LTR on the 3' region contains the functional termination signal. Most retrovirus LTRs also include sequences that can enhance the transcriptional activity of flanking viral or cellular DNA. Immediately contiguous to the LTRs are sequences essential for virus DNA synthesis; in addition, a sequence necessary for packaging of the viral RNA genome into nascent virions at the cell membrane is located near the 5' LTR.

The LTRs control three major regions in the virus genome; gag, pol, and env. The gag region codes for four structural proteins used in encapsidation. These proteins bind to 5' regions of the genome. The pol region codes for the reverse transcriptase, an endonuclease which functions in insertion of the provirus into the chromosome, and an endopeptidase. Env genes code for proteins involved in budding of infectious virus particles from the cell.

The gag, pol, and env regions represent a very intricate system with regulatory regions super-imposed on the structural genes. The functions of these control regions have not yet been fully elucidated. In a retroviral vector, some or

all of the gag, pol, and env genes are deleted and the gene(s) of interest inserted. The major differences between the various research groups currently developing vectors for human gene therapy are the modifications introduced into these regions.

Dr. Anderson said investigators attempting to engineer retroviral vectors face several problems. The major problem is constructing a "workable" vector. The vector must be introduced into cells of the animals' body, and the gene of interest expressed at reasonable levels. At this time, high expression of these vectors has not been obtained in animals. Vectors which should provide improved expression are being developed, but it is not yet known whether these vectors will have high enough expression levels to be useful in human gene therapy. High levels of expression depend on the control regions and to a lesser extent the introduced gene.

Dr. Anderson said the second issue faced by investigators is whether unanticipated untoward effects may occur. The important considerations in evaluating the possibility of untoward effects are described in detail in the "Points to Consider in the Design and Submission of Human Somatic Cell Gene Therapy Protocols" developed by the working group.

Mr. Capron asked whether untoward events were possible with retroviral vectors since basic retroviral functions are not well understood. He asked Dr. Anderson to offer a hypothetical scenario of such an event. Dr. Anderson said a primary conjectural effect would be induction of cancer through insertional activation of cellular proto-oncogenes.

Dr. Anderson reminded the group that gene therapy would be applied to seriously ill individuals, and risk/benefit considerations would have to be balanced. He said risk is inherent in any procedure. He offered the example of David the "bubble boy." David, who was born with severe combined immune deficiency disease, received a bone marrow tranplant from his sister in the hope that the transplant would reconstitute his immune system. However, David died as a result of the unexpected activation of latent EBV virus in the bone marrow cells of the donor.

Dr. Anderson said data generated with animal systems could be used to predict a probability of inducing carcinogenesis through retrovirus mediated insertional activation. He said the risk of injury is expected to be low based on current experience with animal systems.

Dr. George Scangos of Johns Hopkins University said the important issue of insertional activation arises because the site of retrovirus insertion cannot be controlled, and integration could result in activation of a cellular proto-oncogene. Whether the patient's immune system would be able to control a tumor resulting from activation of a proto-oncogene is not known.

Dr. Anderson suggested such tumors would rarely occur in heterozygous individuals. Problems are not evident in heterozygous transgenic mice; serious problems are evident, however, in homozygous descendants.

Dr. Walters asked whether investigators would be able to detect an untoward event within a few weeks of treatment. Dr. Anderson replied they might.

Dr. Motulsky said the Working Group on Human Gene Therapy would evaluate the possibility of patient injury or spread of recombinant viruses to other people; the points to consider document developed by the working group addresses these issues.

Dr. Rich thought at the present time the risks associated with drug therapies can be evaluated with greater certainty than the risks associated with human gene therapy.

Mr. Capron said the current drug evaluation process is also inexact. Some drugs which appear to be very safe with no evident adverse effects have caused problems in patients several years after administration. Similarly, negative side effects of human gene therapy may not be detected for many years; some diseases caused by retroviruses have a long incubation period. Ultimately, only human studies will demonstrate safety in humans.

Ms. Witherby said two issues impacting on an evaluation of the potential for negative effects are: (1) whether test animals have been followed for a sufficiently long period of time; and (2) whether the effects of the therapy could be controlled or are reversible.

Report on the November 12, 1985, Meeting of the Recombinant DNA Advisory Committee (RAC) Working Group on Viruses

Dr. Walters then asked Dr. Gottesman, the Chair of the Working Group on Viruses, to report on the November 12, 1985, meeting of that working group. Dr. Gottesman said the November 12, 1985, meeting was primarily convened: (1) to respond to scientific issues originating in a memorandum from the Department of Health and Human Services; and (2) to address the impact of recent developments in virology on the NIH Guidelines for Research Involving Recombinant DNA Molecules.

Dr. Gottesman said the major issues discussed by the Working Group on Viruses were: (1) should RNA derived from recombinant DNA be explicitly covered by the NIH Guidelines; (2) should the NIH Guidelines be modified with respect to cloning of small fragments of viruses in tissue culture systems; (3) should the NIH Guidelines be modified to explicitly refer to experiments involving retroviruses; and (4) what safety levels are appropriate for research involving retroviruses?

Dr. Gottesman said currently the NIH Guidelines only refer to recombinant DNA and do not refer to RNA derived from recombinant DNA although RNA viruses would appear to be covered by case law. A proposal addressing RNA derived from recombinant DNA was developed by a RAC working group and reviewed by the Working

Group on Viruses. The Working Group on Viruses thought the proposal was appropriate. This proposal will be presented to the RAC for consideration at the January 27, 1986, meeting.

Dr. Gottesman reported that the Working Group on Viruses felt it would be appropriate to modify the NIH Guidelines to exempt tissue culture experiments involving small fragments of viral genomes. An argument for exempting small pieces of viruses is that few mechanisms exist for spread of these small pieces, and a modification exempting tissue culture experiments involving small pieces of viral genomes would reduce the Institutional Biosafety Committee (IBC) workload.

Dr. Gottesman reported that three important issues were raised during the working group discussion of the status of retroviruses under the NIH Guidelines: (1) Is the virus an infectious or a defective virus? An infectious virus is capable of multiple rounds of replication. Defective viruses usually can only go through one round of replication. Recombinant vectors, including retroviral vectors, are generally defective since the added genetic material replaces information necessary for replication. The NIH Guidelines are generally less concerned about the potential hazard associated with defective viruses. Retroviruses recombine at high frequency, however; and this aspect of their behavior may permit them to generate infectious particles through recombination with helper viruses or endogenous retroviral sequences. The consensus of the group was that while recombination frequency in retroviruses is high, the probability of recombination and the products of such recombinations can generally be predicted. (2) What is the host range of the retrovirus and the derived vector? Some retroviruses have a narrow host range; other retroviruses have a broad host range. Those retroviruses possessing a broad host range present a greater concern. (3) What are the effects of the retroviruses or the derived vectors on the host? For example, is the virus oncogenic or cytotoxic?

Dr. Gottesman said the discussion on appropriate safety levels for research involving retroviruses indicated that most laboratories use Biosafety Level (BL) 2 containment for research involving retroviruses. Some laboratories in addition employ BL3 practices for research with certain viruses. Dr. Gottesman said the Office of Recombinant DNA Activities (ORDA) currently recommends BL2 conditions for research involving retroviral agents.

Dr. Gottesman said no clear consensus emerged at the November 12, 1985, meeting on how the NIH Guidelines should deal with retroviruses or retroviral vectors. All working group members, however, felt some combinations of characteristics should be carefully evaluated. Dr. Gottesman suggested experiments involving retroviruses could be performed under BL2 containment. However, if the vector possesses two of the three characteristics of infectivity, oncogenicity and broad host range, consideration should be given to rising containment to BL3.

Dr. Robert Cook-Deegan of the Office of Technology Assessment asked how Rous sarcoma virus (RSV) would be treated under this proposal. RSV is infectious and oncogenic, but because its host range does not include humans it is not considered a danger to investigators.

Dr. Anderson said a virus should probably possess the three characteristics of infectivity, oncogenicity, and broad host-range before BL3 containment is required.

Dr. Gottesman said the working group also addressed the issue of whether Appendix B, "Classification of Microorganisms on the Basis of Hazard," of the NIH Guidelines should be modified. Appendix B does not classify retroviruses although other types of viruses are classified in this appendix. The working group discussion indicated a preference for language describing the considerations involved in using retroviruses rather than a specific classification of retroviruses in Appendix B of the NIH Guidelines. They felt descriptive language might be more flexible than a listing in Appendix B.

Dr. Walters said a portion of the November 12, 1985, meeting of the Working Group on Viruses was devoted to the vectors to be used in human gene therapy. The working group discussed: (1) vector recombination, and (2) insertional activation or inactivation of chromosomal genes. Dr. Walters said the consensus was that the "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" adequately address these issues.

Dr. Gottesman said the Working Group on Viruses agreed insertional activation or inactivation by human gene therapy vectors are low probability events. They noted, however, that high levels of recombination occur with retroviruses, and this high level of recombination should be considered in developing human gene therapy vectors. Dr. Grobstein said a fourth item of concern may exist, activation of chromosomal genes which are not proto-oncogenes through retrovirus insertion.

Dr. Motulsky said many aspects of retroviral biology are unknown; he questioned whether human gene therapy vectors might produce surprises. He offered the example of HTLV III/LAV; at this time it is not known why certain T-cells are infected and destroyed by the virus while in other patients neural tissue is the target.

Dr. Anderson said HTLV III/LAV has a regulatory system different from that of other retroviruses; the tat gene of HTLV III/LAV is a post-transcriptional activator and apparently activates translation of both its own and the products of other genes. Dr. Gottesman agreed that investigators do not know all the rules regulating retroviral behavior.

Mr. Capron asked whether investigators believe the current hypothetical model of retrovirus functioning is correct. Dr. Anderson felt the model is probably correct since investigators can generally predict the results of experiments based on this model.

Transgenic Animal Studies

Dr. Walters said Dr. Scangos would describe transgenic experiments involving the introduction of foreign DNA into animal genomes.

Dr. Scangos said transgenic animals are made for several reasons: (1) to study tissue and stage-specific gene expression in development; (2) to study phenotypic effects of foreign gene expression; (3) to study oncogene expression; (4) to study insertional mutagenesis; and (5) to produce animal models of human disease. In the future, a sixth reason may be to create transgenic "factory" animals which would produce medically important compounds.

Dr. Scangos said there are four methods of introducing foreign DNA into animals: (1) microinjection of foreign DNA into the blastocoel cavity of early embryos; (2) exposure of early embryos to an infectious retrovirus; (3) transfecting DNA into totipotent teratocarcinoma cells followed by injection of selected cells into the blastocoel; alternatively, nuclei from such cells can be introduced into fertilized eggs from which the pronuclei have been removed; and (4) microinjection of DNA directly into the pronuclei of fertilized eggs.

Dr. Scangos said microinjection of DNA into the pronuclei of fertilized eggs is the technique most used for introducing foreign DNA. Microinjection allows a variety of different DNA molecules to be introduced into the cells of the experimental animals; there is no constraint on the size or the sequence of the DNA to be introduced. Superovulated female mice are mated, and the one-cell embryos of the pronuclear stage are removed from the reproductive tract. The one-cell embryos are stabilized against a blunt-end glass pipette and foreign DNA injected through a fine glass needle into one of the two pronuclei. Approximately 50% of the injected embryos are lost at this point. The surviving microinjected embryos are then implanted in the oviducts of pseudopregnant female mice. Ten to 20% of the implanted embryos develop into mice. Twenty-five percent of these offspring will have incorporated the foreign gene(s) into their genomes. A subset of the animals incorporating the gene into their chromosomes express the foreign gene. The success rate of this technique in obtaining mice expressing the foreign gene is approximately 1 to 3% depending on the season and the mouse strain.

Dr. Scangos said the injected DNA most often integrates into a single chromosomal site; the site of insertion cannot be controlled experimentally. Multiple copies of the injected DNA are typically arranged in the chromosomal insertion site in tandem head-to-tail arrays containing up to several hundred copies; copy number is not under experimental control. The mechanism of integration is not known; the foreign DNA might be incorporated either by recombination or during chromosome repair. Microinjected DNA appears to integrate randomly at any site in the genome including the middle of genes. How multiple copies of the microinjected DNA integrate into the chromosome at a single site is not known. Since multiple copies of the foreign gene are injected into the embryo, one possibility is that one copy of the injected gene may integrate into a chromosomal site; and other copies may subsequently integrate by homologous recombination with the original copy. The other possibility is that long chains of copies of the injected gene form in the cell and subsequently integrate into a single chromosomal site.

Dr. Gorovitz asked whether substances such as restriction enzymes are introduced into the embryos during microinjection. Dr. Scangos replied that only the foreign DNA is injected into the embryos.

Dr. Scangos said transgenic mice usually express foreign genes in a tissue specific pattern. The factors regulating tissue specific expression are not understood, although expression appears to depend on the site of chromosomal insertion with overall regulation of expression determining in which tissues the genes will be expressed. Chromosomal domains appear to have an effect on the level, but do not appear to affect tissue specific expression. It appears that a 200 base pair sequence contiguous with the promotor at the 5' end of the rat elastase 1 gene directs expression specifically to pancreatic tissue.

Dr. Scangos offered some examples of microinjected foreign genes which were expressed in a tissue specific pattern; the rat elastase 1 gene was expressed in pancreatic acinar cells; the human beta-globin gene was expressed in erythroid cells; a mouse/human beta-globin hybrid gene was expressed in erythroid cells; the rat myosin light chain gene was expressed in skeletal muscle; the mouse alphafetoprotein gene was expressed in yolk sac and liver; the mouse kappa light chain (Ig) gene was expressed in B cells; and the mouse mu heavy chain (Ig) was expressed in B and T cells. Levels of expression of these proteins vary in transgenic animals. For example, rat elastase 1 was expressed at a level 1200 percent above endogenous expression; however, only two percent more mouse/human beta-globin was expressed. Gene expression levels vary from mouse to mouse and from gene to gene. The level of expression does not usually correlate with gene copy number.

Dr. Scangos said tumors arising in trangenic animals microinjected with oncogenes ligated to tissue specific regulatory sequences termed "enhancers" appear to be tissue type specific. He then described some of the types of tumors which arise in transgenic individuals. Choroid plexus papillomas develop within six months of birth in mice microinjected with the SV40 virus; pancreatic adenoma results from microinjection of a hybrid gene composed of SV40 sequences and the rat elastase 1 gene; insulinoma results from microinjection of a hybrid gene composed of SV40 sequences and the gene for insulin; hepatocellular carcinoma and insulinoma result from microinjection of a hybrid gene composed of SV40 sequences and sequences of the virus MT (SV40-MT), additional pathology observed in the offspring of animals injected with SV40-MT are peripheral neuropathy and abnormal myelination; mammary carcinoma are observed in lactating females microinjected with the hybrid gene composed of enhancer sequences of mouse mammary tumor virus (MMTV) and the myc gene (MMTV-myc); adrenal neuroblastoma are observed in transgenic individuals expressing JC virus genes, additional observed pathology are central nervous system neuropathy and abnormal myelination.

The enhancer sequences are implicated in directing tumor development in microinjected animals to certain types of tissues; the tumors do not occur in tissues which would not normally express the gene. For example, transgenic mice bearing the myc gene fused to the enhancer elements of MMTV develop mammary tumors

during the second or third pregnancy. Tumors did not develop in other tissues even though the MMTV-myc genes were expressed in other tissues.

Dr. Scangos said a high percentage of the animals expressing these genes transmit the effect to their progeny.

Dr. Scangos said insertional mutagenesis occurs in regions essential to viability in transgenic animals at a fairly high frequency. The effects of insertional mutagenesis are usually not evident in heterozygous animals. When transgenic animals are inbred, homozygous offspring display these lethal mutations. The available data suggest that 20% of transgenic mice may harbor recessive insertional mutations of essential genes.

Three phenotypes observed with insertional mutagenesis in homozygous animals are: (1) embryonic death; (2) male sterility; and (3) limb deformity. One embryonic lethal mutation arose from insertion of murine leukemia virus in the alpha 1 collagen gene.

Dr. Motulsky asked if there are any examples of a heterozygous dominant arising from insertional mutagenesis. Dr. Scangos said he had heard of a case in which the product of the introduced gene was toxic.

Dr. Scangos said transgenic animals have provided two disease models: (1) transgenic animals obtained through microinjection of the gene coding for the hepatitis B surface antigen mimic chronic carriers of hepatitis B; and (2) animals expressing the JC virus mimic the pathology of progressive multifocal leukoencephalopathy.

Mr. Capron said the human growth hormone gene has been microinjected into animals; he asked Dr. Scangos to comment on these experiments. Dr. Scangos said the human growth hormone gene had been microinjected into sheep, pig, mice, and rabbit embryos. The pigs expressed the gene; however, they were not affected by expression of the human growth hormone gene and grew to normal size. The mice also expressed the gene and grew to larger than normal size.

Dr. Scangos then described the experiments in which the human growth hormone gene was microinjected into mice. The human growth hormone gene was engineered to be under the control of the regulatory sequences of a metallothionein gene. The metallothioneins bind heavy metals such as copper, zinc, cadmium, and mercury and the expression of these genes is increased by the presence of heavy metals in the diet. The diets of mice microinjected with the human growth hormone gene were supplemented by zinc. The mice expressed the human growth hormone gene in tissues that normally synthesize metallothionein and grew to more than normal size; however, since the normal feedback control mechanisms were bypassed, the animals expressed many secondary effects such as pituitary abnormalities, abnormal liver function, and female infertility. The investigators injected subsequent groups of embryos with a fusion gene composed

of a metallothionein promotor and the structural gene for growth hormone releasing factor. This fusion gene also stimulates growth of mice but by a different mechanism through elevation of endogenous growth hormone production. Some of the side effects of excess growth hormone production were alleviated by this strategy.

Mr. Capron asked whether microinjected transgenic animals which do not pass the foreign gene to their offspring have been observed. Dr. Scangos said some animals do not pass the foreign gene to their offspring; whether the gene will be passed to progeny depends on the time at which the microinjected DNA integrates into the chromosomal DNA. If chromosomal integration occurs at the pronuclear stage, the gene will be passed to offspring since all cells will contain the insert. If the gene does not integrate until several cell divisions have occurred, the gene will not be present in all the cells of the body and may not be contained in germ line tissue. The gene then will not be passed to offspring.

Dr. Scangos said cells in tissue culture have also been microinjected with foreign DNA. More than half of the cells surviving microinjection express the injected gene(s).

Dr. Scangos said when retroviruses are used to produce transgenic mice, generally only single copies of the viral DNA integrate and integration occurs at a single site within the chromosomal DNA. The insertion site is not under experimental control. Retroviral infection is usually initiated at later embryonic stages resulting in mosaic founder animals; outbreeding of the founder animal is therefore necessary to establish pure lines hemizygous for a single insertion site. Few embryos are lost when foreign DNA is introduced by retrovirus infection. An efficiency of approximately 25% is possible with viral vectors when they are used to infect preimplantation embryos.

Dr. Motulsky said use of microinjection techniques would not be ethically feasible in human subjects. The tremendous embryo losses associated with the microinjection technique would not be acceptable. In addition, it is not possible to determine whether the egg is normal at the pronuclei stage. For most human recessive diseases, three fertilized eggs out of four would be normal for the trait in question. In order for all the fertilized eggs to possess the recessive trait, both mates would have to be homozygous for the deleterious trait. In humans, such a situation is highly unusual.

Dr. Mahoney suggested the embryo could be tested at the 8 cell stage when one cell could be sacrificed to test for the homozygous presence of the deleterious gene. If some cells were modified at early embryonic stages, an individual mosaic for the trait could develop. In humans, mosaic individuals frequently do not display disease symptoms although they are carriers.

Dr. Anderson said the microinjection technique is only successful at the pronuclear stage of development. The cell is designed to keep DNA out of the nuclei once the pronuclei fuse.

The working group agreed transgenic germ line modifying procedures are not feasible or acceptable for humans at this time.

The FDA IND Process

Dr. Walters asked Dr. Samuel Ackerman of the Bureau of Drugs and Biologics of the FDA to describe to the working group the process of filing a "Notice of Claimed Investigational Exemption for a New Drug (IND)."

Dr. Ackerman said biologics are defined as materials that come from the blood, except for hormones, and materials that come from microorganisms. The Bureau of Biologics is responsible for products such as albumin, viral vaccines, and bacterial vaccines. The Bureau also has jurisdiction over a number of products produced by biotechnology such as monoclonal antibodies, cytokines, and anti-HTLV III test kits.

Dr. Ackerman said the Bureau of Drugs and Biologics is responsible for regulating investigational stages in drug testing, commercial approval of drugs, and periodic examination of products. An IND (Attachment III) must be filed if any of the products used in a procedure are transported across state lines.

Dr. Ackerman said the IND mechanism primarily monitors clinical trials where the major goal is to demonstrate product safety and efficacy. When clinical trials demonstrate safety and efficacy, the product can be licensed, and the commercial production phase begun. The FDA is then responsible for reviewing the integrity of the production process, the facility, and any changes in the manufactured product.

Dr. Ackerman said the IND is keyed to the product and not to the process or the investigator. Indeed, several different investigational groups can file a single IND. The IND is confidential; its existence cannot be divulged, although a Freedom of Information request can be filed to obtain certain information.

Dr. Ackerman then described the information requested by the IND form. Items one through five deal with the preparation and manufacture of the product, and address issues such as identity, components, source, preparation, purity, strength, reproducibility, stability of material, etc. The sixth item requests all available information derived from preclinical investigations, clinical studies, and experience with the drug. Items seven through ten deal with the proposed clinical investigation. Item seven requests an accurate description of prior investigations and experiences and results pertinent to the safety and efficacy of the drug under the conditions of the investigation; it also requests a description of all relevant hazards, contraindications, side-effects, and precautions suggested by prior investigations and experience with the drug or related drugs. The eighth item requests a description of the scientific training and experience considered appropriate to qualify the investigators or suitable experts to investigate the drug or biologic. The ninth item requests the names, training,

and experience of each investigator. The tenth item requests an outline of any phase or phases of the planned investigations and a description of the Institutional Review Board (IRB). The eleventh item requests an agreement that the investigator will notify the FDA if the investigation is discontinued and the reason for discontinuance. Items 12 through 16 deal with notification issues and environmental assessments.

Dr. Ackerman said investigators must file an additional form (Attachment IV) with the FDA as part of an IND application. This form requests information on the principal investigators' qualifications and experience, the requirements of the IRB, and the investigational plan. Item four specifically links the principal investigator to the sponsoring organization which is linked to the FDA and requires that progress reports be made to the FDA at intervals of time not exceeding one year. Any adverse effect that may be regarded as caused by or probably caused by the new drug shall be reported to the sponsor promptly. If the adverse effect is alarming, it shall be reported immediately. Dr. Ackerman said promptly is defined as within seven to ten days and immediately by telephone call.

Mr. Capron asked for the FDA definition of "adverse effect." Dr. Ackerman said the definition of adverse effect is in a constant state of evolution. One camp believes that any effect occurring within thirty days of administration of a drug should be reported. The other camp believes the only effects which need to be reported are those that could with a high degree of confidence be related to the drug. In practice in the beginning stages of a clinical trial, almost every effect is reported; as experience with the drug is gained, fewer observations are reported.

Dr. Gorovitz asked how FDA defines adequacy and safety; some drugs are not safe but are used in desperate cases. Dr. Ackerman replied that adverse effects of a drug are evaluated on a risk/benefit basis. The cases can be highly specific.

Dr. Cook-Deegan asked what criteria trigger FDA involvement in the development of a drug. Dr. Ackerman said FDA can interact at several levels and can issue: regulations; guidelines binding in the legal sense; or points to consider which are less binding and more of an exchange of views. The points to consider documents list FDA concerns and are generally documents in evolution. FDA is considering generating a points to consider document for human gene therapy. A number of triggers exist for initiating such an FDA action. For example, the number of individuals who are involved in the area is one such trigger; another trigger is the amount of controversy surrounding a procedure.

Dr. Ackerman said the FDA attempts to anticipate problems but will not adopt review procedures based on conjecture since such procedures may ultimately impede the review process. Although FDA would like to have some basis for discussion, some experience is needed to develop a reasonable document.

Dr. Motulsky asked how FDA would treat studies such as the National Institutes of Health (NIH) study involving interleukin 2 (IL-2) activation of cancer patient T-cells. Dr. Ackerman replied that procedure involves an autologous

transplant of bone marrow cells treated with a biologic and as such falls under FDA jurisdiction.

Mr. Capron asked whether FDA regulates a single investigator treating a single patient. Dr. Ackerman said in some instances, FDA regulates the individual researcher; human gene therapy may be one such instance. Institutions such as the funding agency, the IRB, the medical school, or the pharmacy compounding the substances used in a protocol also may require the investigator to file an IND. FDA welcomes voluntary IND filings and will encourage investigators to file for human gene therapy protocols.

Dr. Cook-Deegan asked how FDA would proceed on receipt of an IND on human gene therapy. Dr. Ackerman said FDA would first perform a cursory examination of the IND for completeness; FDA scientific staff would evaluate the IND with regard to safety issues and would also examine the manufacturing process. The IND would be routed through the FDA review divisions and the relevant scientific divisions. The scientific divisions include virology, blood and blood products, vaccines, biochemistry and biophysics, and quality control.

Dr. Mahoney asked whether the Working Group on Human Gene Therapy and the FDA could become involved in a jurisdictional dispute. Dr. Walters said the working group and RAC have established a process for review of NIH funded protocols.

Dr. Mahoney asked whether a duplication of effort may occur. Ms. Areen thought the working group and FDA would not perform duplicative reviews since the two groups will be evaluating proposals from two different perspectives. Dr. Walters agreed; he pointed out that FDA will handle submissions in a confidential manner. The NIH working group is part of a public process. In this sense, the two groups will perform different functions. Dr. Gorovitz also agreed that different types of issues will be raised by the two groups; the NIH has its agenda while the FDA has its own interests.

Dr. Mahoney asked whether one group might approve of a protocol and the other group disapprove. Dr. Ackerman said the FDA frequently reviews clinical trials developed by the NIH. No problems in coordinating these reviews has arisen in the past. Dr. Bonnie Lee of the FDA said NIH sponsored research involving FDA approved substances currently is reviewed by both the NIH and the FDA. Such review is complementary.

Mr. Capron suggested the NIH process could serve to alert investigators to FDA requirements and procedures.

Dr. Anderson said investigators might wish to submit partial information to FDA in a confidential process rather than wait until a complete protocol is ready for submission. He asked if an informal cooperative discussion process would be useful to the FDA. Dr. Ackerman said there are a variety of mechanisms for communicating with the FDA short of a formal IND submission; e.g., FDA can arrange pre-IND meetings or conferences. He felt these confidential mechanisms would be useful to investigators contemplating human gene therapy.

Dr. Motulsky said FDA oversight is required at the development and commercialization stages. He questioned, however, whether FDA oversight was necessary for early experimental stages of a protocol. He argued that FDA oversight would not be necessary for this stage if the NIH and the RAC working groups were providing oversight.

Ms. Areen said FDA is constrained by law to regulate certain procedures and must adhere to these statutes. Mr. Capron said FDA may be able to proceed with greater rapidity than NIH in reviewing human gene therapy protocols.

Dr. Gorovitz thought the proposals submitted to FDA and to NIH for review could employ an identical format, but the substantive evaluation of the proposals would be different in the agencies. Dr. Ackerman said although the issues may be the same, the emphasis will not be the same; and the reviews would not be well served by use of an identical format.

Dr. Anderson said the Working Group on Human Gene Therapy attempted in its Points to Consider document to take account of the points addressed in an FDA review; the concepts included in the working group document correspond to the concepts in an IND.

Dr. Gartland asked if FDA either approves or disapproves of an IND. Dr. Ackerman replied that INDs are either active or inactive; the IND is related to the product not to the particular study.

Dr. Gartland asked what type of action FDA could take if an investigator proceeds in the absence of FDA approval. Dr. Ackerman said FDA could inactivate the IND and disqualify the investigator from participation in clinical protocols. FDA might also take legal action. Ms. Areen added that subjects participating in a protocol with an inactive IND could sue the investigator.

Other Issues

Dr. Walters informed the working group that President Reagan's veto of the NIH appropriations bill had been overridden by the Senate. This bill contains language for the establishment of a "Biomedical Ethics Board." One of the first studies of this board will be human applications of genetic technologies.

Dr. Walters also announced the procedure the NIH and the Working Group on Human Gene Therapy would follow in dealing with consent forms. He said the NIH does not generally review consent forms since the Office of Protection Against Research Risks (OPRR) is too small to review all the forms associated with NIH funded protocols. Nonetheless, OPRR does review approximately 200 forms a year. The NIH would not prevent the working group from requesting consent forms for the earliest gene therapy protocols, and the working group will request and review these forms.

Ms. Witherby thought the consent form would have an important impact on the working group's decision. Mr. Capron thought it would be a disservice to the NIH not to review the consent form since in the absence of such review the NIH cannot be assured it is providing the appropriate level of care. Dr. Ackerman said the FDA will probably request submission of the consent form as part of the IND process for human gene therapy protocols.

Dr. Walters said one issue the working group should address is the sequence of review; would a protocol require local IBC and IRB approval before submission to the working group.

Dr. Motulsky thought it illogical that the groups which have the least experience with human gene therapy, the local IRBs and IBCs, are the groups which are required to perform the first reviews. Dr. Gottesman said the points to consider document indicates IRBs and IBCs may condition approval on RAC approval.

Dr. Gottesman said one suggestion the Working Group on Viruses considered was whether NIH could certify vectors for use in human gene therapy protocols. While she did not think vector certification would speed up the review process, she suggested the Working Group for Human Gene Therapy could extend to investigators the option of submitting preclinical data on vector construction for review before the clinical protocol is submitted.

Ms. Areen thought the working group should not delay IBC review of protocols for a working group dialogue on vectors. Dr. Grobstein did not think the working group should review preclinical data independent of the protocol; the vectors should be considered in the context of the whole protocol. Dr. Mahoney said he would not wish this process to be seen as a negotiating process between investigators and the working group. Dr. Childress thought the working group process should encourage an information exchange but should not approve portions of experiments.

Dr. Walters said the working group could indicate review of vector data is part of the working group self-education effort. Dr. Anderson suggested a memorandum indicating this position should be published in the Federal Register in order to alert investigators to the working group's position. Ms. Areen suggested working group memoranda could also alert IBCs and IRBs of the working group's position.

Dr. Walters then asked the working group to consider the scheduling of the next working group meetings. He said the next RAC meetings are scheduled for January 27, 1986; May 12, 1986; and September 29, 1986. He suggested the working group schedule their meetings to coincide with the RAC meeting schedule.

Dr. Gartland said ORDA would telephone the members of the working group in order to select meeting dates. He offered the working group a schedule (Attachment V) showing the deadlines for proposal submission prior to a RAC meeting.

Dr. Grobstein suggested a more detailed discussion of the time-line for scheduling meetings might be held at the next working group meeting in light of clincians' experience with clinical trials.

Dr. Gottesman said the working group might at some meeting address the issue of what constitutes a minor modification of a protocol.

Dr. Motulsky suggested the working group also discuss how confidential information should be handled; he could imagine cases where confidential information disclosed in one proposal might affect review of other proposals.

Ms. Areen suggested the working group might consider how the group would proceed on receipt of a report of a harmful effect. Would the working group be called into session to address the issue, the impact of the harmful effect on the protocol, and the impact on other protocols?

Dr. Walters asked the working group whether they would approve of the formation of a subgroup to develop a document describing in lay terminology the purpose of the points to consider document and the working group. The second issue which could be evaluated is whether the points to consider have reached the appropriate audience.

Ms. Witherby said she would like to see a "lay" statement describing the working group's agenda. She agreed to attempt to write a first draft for such a statement.

Dr. Walters adjourned the meeting of the Working Group on Human Gene Therapy at 3:35 p.m. on December 16, 1985.

Respectfully submitted,

Elizabeth Milewski

Elizabeth A. Milewski, Ph.D.
Rapporteur

William J. Gartland, Jr., Ph.D.
Executive Secretary

I hereby certify that, to the best of my knowledge, the foregoing Minutes and Attachments are accurate and complete.

5/12/86
Date

LeRoy Walters

LeRoy Walters, Ph.D.
Chair

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WORKING GROUP ON HUMAN GENE THERAPY

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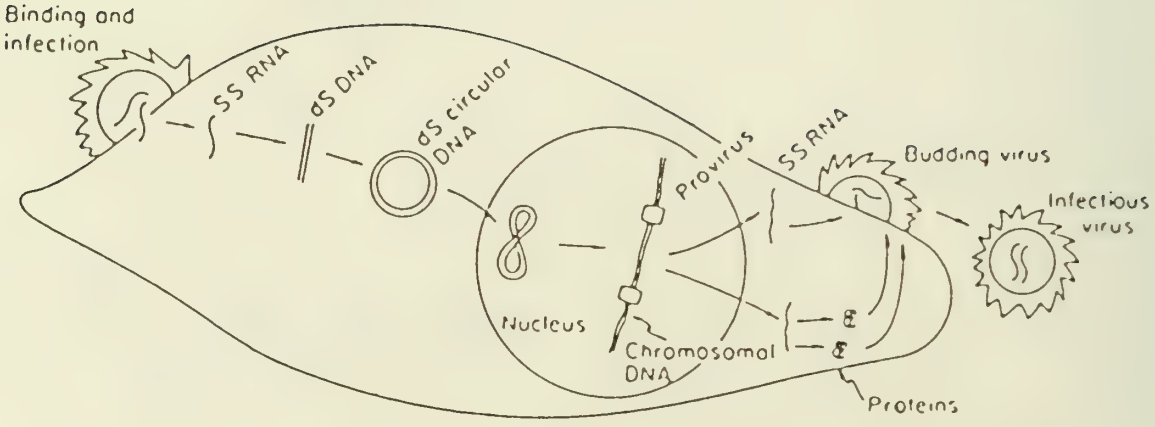
DECEMBER 1985



From: Gene Transfer with Retrovirus Vectors. Bernstein, A., S. Berger, D. Huszar, and J. Dick. 1985. In Genetic Engineering: Principles and Methods, Vol. 7. (Ed. J. K. Setlow and A. Hollaender). Plenum Publishing Corporation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

Form Approved; OMB No. 0910-0014
Expiration Date: February 29, 1984.

NOTICE OF CLAIMED INVESTIGATIONAL EXEMPTION FOR A NEW DRUG

NOTE: No drug may be shipped or study initiated unless a complete statement has been received. *(21 CFR 312.1(a)(2)).*

Name of Sponsor _____ Date _____

Address _____ Telephone ( ) _____

Name of Investigational Drug _____

FOR A DRUG:

Food And Drug Administration
Office of New Drug Evaluation *(HFN-106)*
5600 Fishers Lane
Rockville, Maryland 20857

FOR A BIOLOGIC:

Food and Drug Administration
Office of Biologics *(HFN-823)*
8800 Rockville Pike
Bethesda, Maryland 20205

Dear Sir:

The sponsor, _____, submits this notice of claimed investigational exemption for a new drug under the provisions of section 505(i) of the Federal Food, Drug, and Cosmetic Act and § 312.1 of Title 21 of the Code of Federal Regulations.

Attached hereto in triplicate are:

1. The best available descriptive name of the drug, including to the extent known the chemical name and structure of any new-drug substance, and a statement of how it is to be administered. (If the drug has only a code name, enough information should be supplied to identify the drug.)

2. Complete list of components of the drug, including any reasonable alternates for inactive components.

3. Complete statement of quantitative composition of drug, including reasonable variations that may be expected during the investigational stage.

4. Description of source and preparation of, any new-drug substances used as components, including the name and address of each supplier or processor, other than the sponsor, or each new-drug substance.

5. A statement of the methods, facilities, and controls used for the manufacturing, processing, and packing of the new drug to establish and maintain appropriate standards of identity, strength, quality, and purity as needed for safety and to give significance to clinical investigations made with the drug.

6. A statement covering all information available to the sponsor derived from preclinical investigations and any clinical studies and experience with the drug as follows:

a. Adequate information about the preclinical investigations, including studies made on laboratory animals, on the basis of which the sponsor has concluded that it is reasonably safe to initiate clinical investigations with the drug: Such information should include identification of the person who conducted each investigation; identification and qualifications of the individuals who evaluated the results and concluded that it is reasonably safe to initiate clinical investigations with the drug and a statement of where the investigations were conducted and where the records are available for inspection; and enough details about the investigations to permit scientific review. The preclinical investigations shall not be considered adequate to justify clinical testing unless they give proper attention to the conditions of the proposed clinical testing. When this information, the outline of the plan of clinical pharmacology, or any progress report on the clinical pharmacology, indicates a need for full review of the preclinical data before a clinical trial is undertaken, the Department will notify the sponsor to submit the complete preclinical data and to withhold clinical trials until the review is completed and the sponsor notified. The Food and Drug Administration will be prepared to confer with the sponsor concerning this action.

b. If the drug has been marketed commercially or investigated (e.g. outside the United States), complete information about such distribution or investigation shall be submitted, along with a complete bibliography of any publications about the drug.

c. If the drug is a combination of previously investigated or marketed drugs, an adequate summary of preexisting information from preclinical and clinical investigations and experience with its components, including all reports available to the sponsor suggesting side-effects, contraindications, and ineffectiveness in use of such components: Such summary should include an adequate bibliography of publications about the components and may incorporate by reference any information concerning such components previously submitted by the sponsor to the Food and Drug Administration. Include a statement of the expected pharmacological effects of the combination.

d. If the drug is a radioactive drug, sufficient data must be available from animal studies or previous human studies to allow a reasonable calculation of radiation absorbed dose upon administration to a human being.

7. A total (one in each of the three copies of the notice) of all informational material, including label and labeling, which is to be supplied to each investigator: This shall include an accurate description of the prior investigations and experience and their results pertinent to the safety and possible usefulness of the drug under the conditions of the investigation. It shall not represent that the safety or usefulness of the drug has been established for the purposes to be investigated. It shall describe all relevant hazards, contraindications, side-effects, and precautions suggested by prior investigations and experience with the drug under investigation and related drugs for the information of clinical investigators.

8. The scientific training and experience considered appropriate by the sponsor to qualify the investigators as suitable experts to investigate the safety of the drug, bearing in mind what is known about the pharmacological action of the drug and the phase of the investigational program that is to be undertaken.

9. The names and a summary of the training and experience of each investigator and of the individual charged with monitoring the progress of the investigation and evaluating the evidence of safety and effectiveness of the drug as it is received from the investigators, together with a statement that the sponsor has obtained from each investigator a completed and signed form, as provided in subparagraph (12) or (13) of this paragraph, and that the investigator is qualified by scientific training and experience as an appropriate expert to under-

take the phase of the investigation outlined in section 10 of the "Notice of Claimed Investigational Exemption for a New Drug." (In crucial situations, phase 3 investigators may be added and this form supplemented by rapid communication methods, and the signed Form FD-1573 shall be obtained promptly thereafter.)

10. An outline of any phase or phases of the planned investigations and a description of the institutional review committee, as follows:

a. Clinical pharmacology. This is ordinarily divided into two phases: Phase 1 starts when the new drug is first introduced into man - only animal and in vitro data are available - with the purpose of determining human toxicity, metabolism, absorption, elimination, and other pharmacological action, preferred route of administration, and safe dosage range; phase 2 covers the initial trials on a limited number of patients for specific disease control or prophylaxis purposes. A general outline of these phases shall be submitted, identifying the investigator or investigators, the hospitals or research facilities where the clinical pharmacology will be undertaken, any expert committees or panels to be utilized, the maximum number of subjects to be involved, and the estimated duration of these early phases of investigation. Modification of the experimental design on the basis of experience gained need be reported only in the progress reports on these early phases, or in the development of the plan for the clinical trial, phase 3. The first two phases may overlap and, when indicated, may require additional animal data before these phases can be completed or phase can be undertaken. Such animal tests shall be designed to take into account the expected duration of administration of the drug to human beings, the age groups and physical status, as for example, infants, pregnant women, premenopausal women, of those human beings to whom the drug may be administered, unless this has already been done in the original animal studies. If a drug is a radioactive drug, the clinical pharmacology phase must include studies which will obtain sufficient data for dosimetry calculations. These studies should evaluate the excretion, whole body retention, and organ distribution of the radioactive material.

b. Clinical trial. This phase 3 provides the assessment of the drug's safety and effectiveness and optimum dosage schedules in the diagnosis, treatment, or prophylaxis of groups of subjects involving a given disease or condition. A reasonable protocol is developed on the basis of the facts accumulated in the earlier phases, including completed and submitted animal studies. This phase is conducted by separate groups following the same protocol (with reasonable variations and alternatives permitted by the plan) to produce well-controlled clinical data. For this phase, the following data shall be submitted:

i. The names and addresses of the investigators. (Additional investigators may be added.)

ii. The specific nature of the investigations to be conducted, together with information or case report forms to show the scope and detail of the planned clinical observations and the clinical laboratory tests to be made and reported.

iii. The approximate number of subjects (a reasonable range of subjects is permissible and additions may be made), and criteria proposed for subject selection by age, sex, and condition.

iv. The estimated duration of the clinical trial and the intervals, not exceeding 1 year, at which progress reports showing the results of the investigations will be submitted to the Food and Drug Administration.

c. Institutional review board (IRB). The sponsor must give assurance that an IRB that complies with the requirements set forth in Part 56 of this chapter will be responsible for the initial and continuing review and approval of the proposed clinical study. The sponsor must also provide assurance that the investigators will report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and that the investigators will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazard to the human subjects. FDA will regard the signing of the Form FDA-1571 as providing the necessary assurances above.

(The notice of claimed investigational exemption may be limited to any one or more phases, provided the outline of the additional phase or phases is submitted before such additional phases begin. A limitation on an exemption does not preclude continuing a subject on the drug from phase 2 to phase 3 without interruption while the plan for phase 3 is being developed.)

Ordinarily, a plan for clinical trial will not be regarded as reasonable unless, among other things, it provides for more than one independent competent investigator to maintain adequate case histories of an adequate number of subjects, designed to record observations and permit evaluation of any and all discernible effects attributable to the drug in each individual treated, and comparable records on any individuals employed as controls. These records shall be individual records for each subject maintained to include adequate information pertaining to each, including age, sex, conditions treated, dosage, frequency of administration of the drug, results of all relevant clinical observations and laboratory examinations made, adequate information concerning any other treatment given and a full statement of any adverse effects and useful results observed, together with an opinion as to whether such effects or results are attributable to the drug under investigation.

11. A statement that the sponsor will notify the Food and Drug Administration if the investigation is discontinued, and the reason therefor.

12. A statement that the sponsor will notify each investigator if a new-drug application is approved, or if the investigation is discontinued.

13. If the drug is to be sold, a full explanation why sale is required and should not be regarded as the commercialization of a new drug for which an application is not approved.

14. A statement that the sponsor assures that clinical studies in humans will not be initiated prior to 30 days after the date of receipt of the notice by the Food and Drug Administration and that he will continue to withhold or to restrict clinical studies if requested to do so by the Food and Drug Administration prior to the expiration of such 30 days. If such request is made, the sponsor will be provided specific information as to the deficiencies and will be afforded a conference on request. The 30-day delay may be waived by the Food and Drug Administration upon a showing of good reason for such waiver; and for investigations subject to institutional review committee approval as described in item 10c above, and additional statement assuring that the investigation will not be initiated prior to approval of the study by such committee.

15. When requested by the agency, an environmental impact analysis report pursuant to § 25.1 of this chapter.

16. A statement that all nonclinical laboratory studies have been, or will be, conducted in compliance with the good laboratory practice regulations set forth in Part 58 of this chapter, or, if such studies have not been conducted in compliance with such regulations, a statement that describes in detail all differences between the practices used in conducting the study and those required in the regulations.

Very truly yours,

SPONSOR	PER
	INDICATE AUTHORITY

(This notice may be amended or supplemented from time to time on the basis of the experience gained with the new drug. Progress reports may be used to update the notice.)

ALL NOTICES AND CORRESPONDENCE SHOULD BE SUBMITTED IN TRIPLICATE.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

STATEMENT OF INVESTIGATOR

Form approved; OMB No. 0910-0013
Expiration Date: December 31, 1984.

Note: No drug may be shipped or study initiated unless a completed statement has been received *(21 CFR 312.1(a)(12)).*

TO: SUPPLIER OF DRUG *(Name, address, and Zip Code)*

NAME OF INVESTIGATOR *(Print or Type)*

DATE

NAME OF DRUG

Dear Sir:

The undersigned, _____ submits this statement as required by section 505(i) of the Federal Food, Drug, and Cosmetic Act and §312.1 of Title 21 of the Code of Federal Regulations as a condition for receiving and conducting clinical investigations with a new drug limited by Federal *(or United States)* law to investigational use.

1. THE FOLLOWING IS A STATEMENT OF MY EDUCATION AND EXPERIENCE:

a. COLLEGES, UNIVERSITIES, AND MEDICAL OR OTHER PROFESSIONAL SCHOOLS ATTENDED, WITH DATES OF ATTENDANCE, DEGREES, AND DATES DEGREES WERE AWARDED

b. POSTGRADUATE MEDICAL OR OTHER PROFESSIONAL TRAINING. GIVE DATES, NAMES OF INSTITUTIONS, AND NATURE OF TRAINING.

c. TEACHING OR RESEARCH EXPERIENCE. GIVE DATES, INSTITUTIONS, AND BRIEF DESCRIPTION OF EXPERIENCE.

d. EXPERIENCE IN MEDICAL PRACTICE OR OTHER PROFESSIONAL EXPERIENCE. GIVE DATES, INSTITUTIONAL AFFILIATIONS, NATURE OF PRACTICE, OR OTHER PROFESSIONAL EXPERIENCE.

e. REPRESENTATIVE LIST OF PERTINENT MEDICAL OR OTHER SCIENTIFIC PUBLICATIONS. GIVE TITLES OF ARTICLES, NAME OF PUBLICATIONS AND VOLUME, PAGE NUMBER, AND DATE.

IF THIS INFORMATION HAS PREVIOUSLY BEEN SUBMITTED TO THE SPONSOR, IT MAY BE REFERRED TO AND ANY ADDITIONS MADE TO BRING IT UP-TO-DATE.

2a. The investigator assures that an IRB that complies with the requirements set forth in Part 56 of this chapter will be responsible for the initial and continuing review and approval of the proposed clinical study. The investigator also assures that he/she will report to the IRB all changes in the research activity and all unanticipated problems involving risks to human subjects or others, and that he/she will not make any changes in the research that would increase the risks to human subjects without IRB approval. FDA will regard the signing of the Form FDA 1573 as providing the necessary assurances stated above.

b. A description of any clinical laboratory facilities that will be used. (If this information has been submitted to the sponsor and reported by him on Form FDA 1571, reference to the previous submission will be adequate).

3. *The investigational drug will be used by the undersigned or under his supervision in accordance with the plan of investigation described as follows: (Outline the plan of investigation including approximation of the number of subjects to be treated with the drug and the number to be employed as controls, if any; clinical uses to be investigated; characteristics of subjects by age, sex and condition; the kind of clinical observations and laboratory tests to be undertaken prior to, during, and after administration of the drug; the estimated duration of the investigation; and a description or copies of report forms to be used to maintain an adequate record of the observations and test results obtained. This plan may include reasonable alternates and variations and should be supplemented or amended when any significant change in direction or scope of the investigation is undertaken.)*

4. THE UNDERSIGNED UNDERSTANDS THAT THE FOLLOWING CONDITIONS, GENERALLY APPLICABLE TO NEW DRUGS FOR INVESTIGATIONAL USE, GOVERN HIS RECEIPTS AND USE OF THIS INVESTIGATIONAL DRUG:

a. The sponsor is required to supply the investigator with full information concerning the preclinical investigations that justify clinical trials, together with fully informative material describing any prior investigations and experience and any possible hazards, contraindications, side-effects, and precautions to be taken into account in the course of the investigation.

b. The investigator is required to maintain adequate records of the disposition of all receipts of the drug, including dates, quantities, and use by subjects, and if the investigation is terminated, suspended, discontinued, or completed, to return to the sponsor any unused supply of the drug. If the investigational drug is subject to the Comprehensive Drug Abuse Prevention and Control Act of 1970, adequate precautions must be taken including storage of the investigational drug in a securely locked, substantially constructed cabinet, or other securely locked substantially constructed enclosure, access to which is limited, to prevent theft or diversion of the substance into illegal channels of distribution.

c. The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the drug or employed as a control in the investigation.

d. The investigator is required to furnish his reports to the sponsor of the drug who is responsible for collecting and evaluating the results obtained by various investigators. The sponsor is required to present progress reports to the Food and Drug Administration at appropriate intervals not exceeding 1 year. Any adverse effect that may reasonably be regarded as caused by, or probably caused by, the new drug shall be reported to the sponsor promptly, and if the adverse effect is alarming, it shall be reported immediately. An adequate report of the investigation should be furnished to the sponsor shortly after completion of the investigation.

e. The investigator shall maintain the records of disposition of the drug and the case histories described above for a period of 2 years following the date a new-drug application is approved for the drug; or if the application is not approved, until 2 years after the investigation is discontinued. Upon the request of a scientifically trained and properly authorized employee of the Department, at reasonable times, the investigator will make such records available for inspection and copying. The subjects' names need not be divulged unless the records of particular individuals require a more detailed study of the cases, or unless there is reason to believe that the records do not represent actual cases studied, or do not represent actual results obtained.

f. The investigator certifies that the drug will be administered only to subjects under his personal supervision or under the supervision of the following investigators responsible to him,

_____

_____

_____

and that the drug will not be supplied to any other investigator or to any clinic for administration to subjects.

g. The investigator certifies that he will inform any subjects including subjects used as controls, or their representatives, that drugs are being used for investigational purposes, and will obtain the consent of the subjects, or their representatives, except where this is not feasible or, in the investigator's professional judgment, is contrary to the best interests of the subjects.

h. The investigator is required to assure the sponsor that for investigations subject to an institutional review requirement under Part 56 of this chapter the studies will not be initiated until the institutional review board has reviewed and approved the study. (The organization and procedure requirements for such a board as set forth in Part 56 should be explained to the investigator by the sponsor.)

Very truly yours,

Name of Investigator _____

Address _____ Telephone ( ) _____

_____

(This form should be supplemented or amended from time to time if new subjects are added or if significant changes are made in the plan of investigation.)

* U.S.GPO:1984-0-454-394/18778

HUMAN GENE THERAPY PROPOSALS
APPROXIMATE REVIEW SCHEDULE (IN WEEKS)

SELECTED LETTERS AND DOCUMENTS RECEIVED IN THE PERIOD MARCH 1985 TO DECEMBER 1985 RELEVANT TO THE NIH GUIDELINES.

5 Doane St., 4th floor
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(617) 227-8035

COMMITTEE FOR RESPONSIBLE GENETICS

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Donald Comb, Ph.D.
Barry Commoner, Ph.D.
Molly Coye, M.D.
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Ernest Englander, Ph.D.
Rick Engler
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Terri Goldberg
Executive Director

March 20, 1985

Secretary Margaret Heckler
U.S. Department of Health
and Human Services
Washington, DC 20201

Dear Secretary Heckler:

I am writing on behalf of the Executive Council of the Committee for Responsible Genetics (CRG) to express our concern about the proposed "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols" published in the Federal Register on January 22, 1985. We do not believe that the Department of Health and Human Services should issue such a document before there has been public discussion about the desirability and appropriateness of human gene therapy and before hearings have been held on the subject. At present there is no mandate or public policy which defines the differences between conventional therapy and gene therapy, between the various purposes for which gene therapy is intended, and between possible forms of gene therapy.

The Presidential Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioral Research 1982 report, Splicing Life, called for the establishment of an oversight body outside of the National Institutes of Health. This proposed group was to examine the social impacts of human gene therapy through dialogue and debate among scientists, lawyers, clergy, representatives of the public, and federal government officials. According to the report, "The need for an appropriate oversight body is based upon the profound nature of the implications of gene splicing as applied to human beings, not upon an immediate threat of harm. Just as it is necessary to run risks and to accept change in order to reap the benefits of scientific progress, it is also desirable that society have means of providing its 'informed consent,' based upon reasonable assurances that risks have been minimized and that changes will occur within an acceptable range" (p. 5).

Our society does not have a means to give "informed consent" to human somatic-cell gene therapy. The

Commission on Human Applications of Genetic Engineering proposed by Senator Albert Gore and amended by Congress was vetoed by President Ronald Reagan as part of the NIH budget for 1985. The Recombinant DNA Advisory Committee Working Group on Human Gene Therapy has effectively addressed many important scientific and public health questions in the "Points to Consider" document, but the Working Group is not an appropriate body to address ethical and social concerns.

One of the unresolved and fundamental social concerns identified in Splicing Life is the possible use of gene therapy for non-life-threatening conditions. The Report concludes that, "Interventions aimed at enhancing 'normal' people as opposed to remedying recognized genetic defects, are also problematic, especially since distinguishing 'medical treatment' from 'nonmedical enhancement' is a very subjective matter" (p. 3). The Report makes clear that such questions are of profound importance and interest for the public.

The CRG Executive Council recommends that you set aside the "Points to Consider" document for the immediate future and establish a Department of Health and Human Services Commission on Human Gene Therapy, which is independent of the RAC and based on the criteria discussed in the Splicing Life report. Such a Commission should hold open hearings and invite public testimony from a variety of sources. On the basis of the information obtained from such hearings, the Commission should determine whether to proceed with human gene therapy, and, if so within what limitations. If the Commission decides that human somatic-cell gene therapy is appropriate, procedures for the design of gene therapy protocols should be established within the framework of the Commission's decision.

We believe that there is a need for an independent body to consider the fundamental questions raised by human genetic engineering. The CRG considers it essential to establish democratic procedures to provide social guidance in this area of critical public concern.

Sincerely,

Terri Goldberg,
Executive Director

cc: Dr. William Gartland
Senator Albert Gore
Congressman John D. Dingell

PHARMACEUTICAL MANUFACTURERS Association

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JOHN JENNINGS, M. D.
VICE PRESIDENT
SCIENCE AND TECHNOLOGY

April 11, 1985

Director
Office of Recombinant DNA Activities
Building 31, Room 3B10
National Institutes of Health
Bethesda, MD 20205

Dear Sirs:

Re: "Points to Consider in the Design And Submission of Human Somatic-Cell Gene Therapy Protocols"

The Pharmaceutical Manufacturers Association is a nonprofit trade association representing 120 manufacturers of prescription pharmaceuticals, biologicals, in vivo and in vitro diagnostic products. Gene therapy is a therapeutic development which evolves, in part, from the knowledge and extensive experience of pharmaceutical research and development, and as a subject for public comment, it is of concern to the pharmaceutical industry. We, therefore, are pleased to offer the following comments on the recent NIH Federal Register notice entitled "Points to Consider in the Design and Submission of Human Somatic-Cell Gene Therapy Protocols."

The notice provides a balanced overview of and perspective on the short term or practical considerations regarding clinical experimentation in humans involving somatic-cell therapy, as well as the longer term social and ethical implications of research in this area. However, the PMA suggests that, in the preamble, a clear distinction be made between somatic-cell therapy and germ cell therapy. The Congressional Office of Technology Assessment, in its report entitled "Human Gene Therapy -- Background Paper", noted that "...because cells that are used in reproduction are not involved, gene therapy (in somatic cells) is quite similar to other kinds of medical therapy, and does not pose new kinds of risks. When considering gene therapy that does not result in inherited change, the factor that most distinguishes it from other medical technologies is its conspicuousness in

the public eye; otherwise, it can be viewed as simply another tool to help individuals overcome an illness."[1] We would urge a comparable statement be included in the introduction to the "Points to Consider" document.

In addition, PMA suggests that, in the preface, the potential benefits of this therapy also be noted. Somatic-cell diagnosis and potential therapy for human single gene defects such as Lesch-Nyhan disease, muscular dystrophy, cystic fibrosis, etc., represent initial targets of a major new approach for treating genetic diseases. Far broader applications are considered possible if present day early tests demonstrate the workability of this new exciting technique. The science is advancing very rapidly, and there appears to be a general religious and ethical acceptance of this approach for possibly ameliorating human single gene defects.

The "Points to Consider" document provides necessary guidance as to what information is required on the objectives and rationale of proposed research involving the use of recombinant DNA technology in humans. Much of the information requested is comparable to that requested by the FDA in its "Points to Consider" document for products produced by recombinant DNA (rDNA) technology.

On the subject of preclinical studies, the animal species in which the studies are performed should be selected on a compound-by-compound basis, and NIH should not specify that all studies be done in primates. One should not assume that primates are the only animals that will predict toxicity in man. The preclinical studies should be done in any species in which the drug shows pharmacological activity on the theory that, if one can define a pharmacologically active dose, one can also define a toxic dose. Therefore, one should not eliminate other animal studies a priori, for, depending on the gene therapy, a different animal model may be preferable.

Concerning the social issues cited in the "Points to Consider" document, we agree that there must be as full communication as possible among investigators and clinicians for products or procedures developed in the proposed study; however, disclosure should comport with relevant patent laws or regulations governing patent rights and proprietary information. Again, a clear distinction should be made between somatic-cell therapy and germ line therapy. The questions posed in the Social Issues section of the document, standing alone, could create confusion in this

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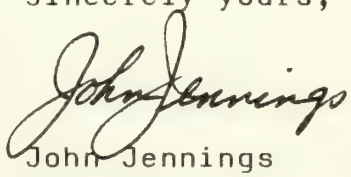
[1] Human Gene Therapy - A Background Paper (Washington, DC: U.S. Congress, Office of Technology Assessment, OTA-BP-BA-32, December 1984)

regard and may lead the public to believe that somatic-cell therapy will necessarily lead to germ line therapy and then possibly state-mandated eugenics. While we recognize that is not the intent of the document, it does raise that spectre and should be clarified.

Our comments are based generally on a PMA position paper on rDNA technology, and a copy of that PMA document is enclosed. It includes a section on the social and ethical issues of human genetic engineering (see Part III), and this section serves as the official PMA statement on this aspect of rDNA technology.

Please pardon our delay in not responding by your February 21 deadline, but we ask that our letter and the section of the PMA position paper on human genetic engineering be submitted as part of the official public record.

Sincerely yours,



John Jennings

Enc.

RECOMBINANT DNA BIOTECHNOLOGY

The Position of the Pharmaceutical Manufacturers Association

July, 1984

TABLE OF CONTENTS

Issue To Be Addressed..... i

Part I. Definition of Biotechnology..... 1

Part II. Guidelines/Regulations of Recombinant DNA Technology..... 4

Part III. The Social and Ethical Issues of Human Genetic Engineering..... 22

Part IV. Balancing Benefits Versus Concerns..... 28

Biotechnology

The Issue to be Addressed

Recent advances in biotechnology, specifically in recombinant DNA (rDNA) technology, offer the pharmaceutical industry the capability and potential for:

(1) improving production efficiencies for existing products;

(2) creating therapeutic and diagnostic products which are found in nature but which otherwise could not be produced economically for commercial use; and,

(3) creating therapeutic and diagnostic products which otherwise could not be synthesized or extracted.

In addition, rDNA technology offers the medical capability and potential for:

(1) intervening genetically in humans to treat known genetic defects; and,

(2) performing in humans other genetic investigations such as genetic engineering experiments.

The first set of advances involve the application of rDNA technologies in microorganisms. The use of the technology in this manner is well under control and differs little from the production of drugs or chemicals by more conventional techniques. However, the second set of advances, biological

"manipulations" in humans now possible by use of techniques such as rDNA technology, are controversial. Their potential human consequences raise questions regarding the socially responsible use of this new technology. What position should the Pharmaceutical Manufacturers Association (PMA) take in order to ensure continued use and development of this technology by the pharmaceutical industry, but at the same time to provide for appropriate guidelines or regulatory controls and a thorough public discussion relative to the socially responsible use of this technology?

This report, in four parts, presents the PMA position. The first part will define biotechnology and discuss some of its potential uses in the pharmaceutical industry. The second part will consider the guidelines and other regulations of rDNA technology in the United States. Part three will deal with the social and ethical questions arising from use of this technology. The final part will balance benefits versus concerns and set forth a Pharmaceutical Manufacturers Association position.

Part I. DEFINITION OF BIOTECHNOLOGY

A. Advances in Biotechnology

Biotechnology refers to the use of living microorganisms, animal and plant cells, or their components such as enzymes or membranes, to transform, synthesize, degrade or concentrate materials. The development of biotechnology processes can involve genetic engineering which is a laboratory-scale operation in which the hereditary material of a cell is altered. The term has come to be applied to a variety of molecular and cellular techniques such as cell fusion or hybridization, transformation, chromosome-mediated gene transfer, and rDNA.

Recombinant DNA, or gene splicing, refers to just one, albeit the most powerful, method of altering the hereditary characteristics of a cell. Genetic engineering alters a cell so that it can produce more or different chemicals or perform better or new functions. Genetic engineering is, therefore, a research technique of great value in the development of new substances for scientific exploration, new products for therapeutic and diagnostic purposes, and improved microorganisms for industrial processes.

The production of proteins by rDNA technology using microorganisms has become an accepted procedure. A long list of proteins including vaccines, hormones, clotting factors, enzymes, bioactive peptides, immunomodulators, etc., has been mentioned and more are constantly added to the list. Future

uses of this new technology are not as clearly defined. Areas that have been mentioned include diagnosis of genetic diseases and food production, e.g., single cell protein and alteration of crop production by manipulating the nitrogen fixation and photosynthetic process. Also, in the energy field, it is hoped to improve current methods of obtaining or utilizing natural gas, or to produce more cheaply, alcohols and other basic organic molecules. Finally, in the chemical field rDNA technology may be used to develop biological systems to replace costly chemical or mechanical conversions.

B. Potential Uses in Pharmaceutical Industry

The first examples of the value of rDNA technology which attracted widespread public attention were in the pharmaceutical field. Genes which code for several human proteins have been cloned in bacteria and the proteins are now produced on a large scale. It is now possible using microbiologically produced human growth hormone to treat pituitary dwarfism without collecting thousands of pituitaries, heretofore the only source of this protein, from autopsies. Interferon, a potential anti-viral and anti-cancer agent, is also being produced through the use of rDNA technology and is undergoing clinical testing in a number of hospitals.

This new technology should shorten the time period between discovery of a protein (or gene product) and clinical application. It offers the medical researcher a tool to amplify

and produce trace compounds so that they might be tested clinically. It is also the only way to obtain species specific compounds such as peptide hormones. Human hormones such as insulin, which in the past could only be obtained with great difficulty, can now be produced on a commercial scale.

Another potential application which holds exciting promise is the creation of synthetic vaccines. Most vaccines are produced from attenuated viruses or bacteria, and though they are generally safe, are not without side effects due to the presence of multiple antigens. Many of these infectious agents have a single protein on their surface which can confer immunity upon the host. The gene for this protein can be cloned and a "single antigen" vaccine can be prepared which offers equal protection but reduced side effects.

This last advance has been made in the veterinary fields as a vaccine against foot and mouth disease was produced using rDNA techniques. A further advantage of this technique is that only the genetic material of the virus needs to be screened. Dangerous viruses can be worked on in small quantities under carefully controlled conditions. This process leads to the cloning of the viral antigen gene away from the remainder of the viral genetic material. The antigen can be produced on a large scale from a completely safe organism, and the product antigen is completely free of viral genetic material and, therefore, is safe to use.

PART II. GUIDELINES/REGULATIONS OF RECOMBINANT DNA TECHNOLOGY

A. Historical Perspective

The United States government was first officially alerted to the potential promise and potential hazards of rDNA technology in 1971 by Dr. James Watson before the House Science and Astronautics Committee (now the Science and Technology Committee). In 1973, during the Gordon Conference on Nucleic Acids in New England, a group of scientists led by Dieter Soll of Yale University, Maxine Singer of the National Cancer Institute, and others, indicated their concern about the potential for a public health hazard from certain types of research involving recombinant DNA molecules. This concern was based upon the realization that the ability to use the newly available restriction and ligating enzymes to cut and recombine the DNA from different species might, in certain types of experiments (such as those involving particularly pathogenic cells or viruses associated with cancer), accidentally produce a recombinant DNA which would code for characteristics that could be hazardous. For example, it was speculated that an inadvertent modification of its DNA might enhance the capability of a seemingly harmless organism so that the altered organism would produce cancer or a highly infectious disease if accidentally or unknowingly released into the environment.

As a result of the concerns expressed by the scientific community, an international meeting was held at Asilomar, California in 1975 and attended by a group of eminent scientists

for the purpose of considering the issue of safety for rDNA technology. From this meeting, the so-called "Asilomar guidelines" for the safe conduct of rDNA research emerged.

The original Asilomar guidelines were formalized in 1976 by the National Institutes of Health (NIH), which had established a special Recombinant DNA Molecule Program Advisory Committee (later renamed the Recombinant DNA Advisory Committee, RAC) to develop guidelines for rDNA work funded by the federal government. After further consideration of the issues and receipt of input from the public and industry representatives, the RAC modified the original Asilomar guidelines and promulgated "NIH Guidelines for Research Involving Recombinant DNA Molecules" which was published in the Federal Register in June 1976. Subsequent revisions and additions provided for voluntary compliance by organizations conducting non-federally funded rDNA research.

The NIH Guidelines resulted from an extraordinary, conscientious effort by scientists, members of the public, and representatives of industry and the federal government, all of who operated in a new, and therefore, unfamiliar realm. The NIH Guidelines appear to be a practical response to concerns in a situation where the risks are mostly hypothetical but where benefits were already becoming apparent. Although significant oversight was imposed, it was possible to proceed in these scientific areas. The guidelines do not deal in any way with other molecular genetic techniques or with the long-term social

or philosophical issues that may be associated with genetic engineering.

The Guidelines have been a dynamic and evolving document which reflects the experience of scientists in the field. As more has been learned about rDNA, the need for less physical containment has been recognized as well as diminshed need for federal oversight.

While activity was occurring at the federal level, intense debate on rDNA research was also taking place at the state and local levels and in universities. As a result of the debate in the late seventies, the most comprehensive state regulations were adopted by Maryland and New York; ordinances or resolutions were passed in Amherst and Cambridge, Massachusetts; Berkeley and Emeryville, California and Princeton, New Jersey. In the early eighties, some states have reacted to the relaxation of federal guidelines by introducing initiatives mandating research compliance with NIH Guidelines. To date, California and Oregon have introduced legislation. While it is too early to predict the outcome of these initiatives, they do indicate a continued interest in rDNA research and development.

B. NIH Guidelines for Research Involving Recombinant DNA Molecules

Basically, the NIH Guidelines provide a set of operating and oversight rules for federally funded rDNA research and

provide a basis for voluntary industry compliance. The NIH Guidelines have come to be viewed by the majority of scientists and policy-makers as a more than adequate means of regulating the conduct of rDNA research. Under the Guidelines, NIH is responsible for the oversight of government-sponsored research involving rDNA molecules. The principal administrative unit established, to help accomplish this objective, has been the Recombinant DNA Advisory Committee (RAC). This group has in the past exercised responsibility for preparing drafts of the Guidelines and, more recently, recommending substantial revisions which downgraded the containment requirement for most experiments. Based on the latest risk assessments, the RAC continues to review the guidelines and offer advice on changes. Beyond those chosen to provide scientific expertise, the membership of the RAC includes individuals knowledgeable in legal, social, environmental and occupational issues but does not include representatives appointed from industry. While one of the current members, David Martin, Jr., M.D., is Vice-President, Research, of Genentech, Dr. Martin was actually appointed while on the faculty of the University of California.

Under the Guidelines, NIH now delegates most of its oversight responsibilities to the institutions which carry on the research by requiring them to establish their own Institutional Biosafety Committees (IBC). The function of these local bodies is to review all rDNA research at the institution

and assure and certify that the Guidelines are being followed. The IBC can issue approval for most types of experiments without prior approval of NIH and even has some limited authority to lower containment levels under specific conditions. According to the NIH Guidelines, IBCs must consist of no less than five members. At least two members are to be persons not affiliated with the institution. Moreover, they should represent the interests of the surrounding community with respect to health and environmental protection.

During its September 1981 meeting, the RAC proposed revisions, based on the report of the Working Group on Revision of the Guidelines, to the existing NIH Guidelines. The major and most controversial feature proposed by the RAC was that the Guidelines would cease to be mandatory and become a voluntary code of standard practices. This proposal triggered moderate scientific and public debate as well as a fair level of media coverage. A separate minority proposal was submitted by Dr. Susan Gottesman of the National Cancer Institute of the NIH, and head of the Working Group on Revision of the Guidelines. This separate proposal outlined relaxation of the Guidelines, which would continue to be mandatory for institutions receiving federal funding. After reviewing the two proposals and evaluating public and scientific comment, the RAC endorsed the Gottesman proposal with minor revisions.

The NIH Guidelines have been amended recently to add Appendix K which sets forth procedures for the control of large

scale experimentation and production using rDNA technology. With this change, the guidelines cover large and small scale uses of rDNA technology in the laboratory and production plant. Overall, the NIH guidelines have worked well and all PMA companies voluntarily comply with them.

In addition to NIH, other federal agencies are examining their existing responsibilities and developing policies with regard to rDNA and other genetic technologies. Thus, industry is in a position to demonstrate responsible action and contribute to the formulation policy.

C. Congress

Congressional activity over the years has reflected the ebb and flow of the public debate. In 1977, several attempts were made to focus attention on the recombinant DNA issues through hearings, legislation, and special evaluations of progress. Proposals were put forth to create a special commission to study the rDNA research issue. At least two committees identified the evolution of this issue over the past several years as a matter of vital public policy concern and suggested the need to monitor the research continuously in order to be prepared for further legislative action if necessary. In 1978, legislation to regulate and study the implications of research involving the rDNA techniques was introduced but never progressed beyond committee evaluation.

Biotechnology, however, remains a vital legislative issue. In 1981 and 1982, the House Science and Technology Sub-

committee on Investigations and Oversight conducted informational hearings on several topics: commercialization of biotechnology, fraud in biomedical research, genetic screening, university and industry cooperation, and application to agriculture research. In April 1983, Representative Albert Gore, Jr., (D-Tenn.) introduced a bill to establish the President's Commission on the Human Applications of Genetic Engineering. The bill (H.R. 2788) was offered and approved as an amendment to the NIH authorization bill in the House Energy and Commerce Committee. The legislation would create a Commission empowered to conduct comprehensive reviews of developments in genetic engineering that have implications for human genetic engineering. The entire NIH bill passed the House in November, 1983. A similar provision has not yet passed the Senate.

D. Federal Agencies and Bodies of Law

In the fall of 1976, a federal interagency committee was established to coordinate regulation of federally-funded rDNA research. Agencies of this committee were: the NIH, National Science Foundation, Department of Agriculture, Veteran's Administration, Department of Energy, Department of Commerce, Department of Defense, Center for Disease Control, Food and Drug Administration, Department of Interior, Department of Justice, Department of Labor, Department of State, Department of Transportation, Environmental Protection Agency, the White

House, NASA, Nuclear Regulatory Commission, and the Arms Control and Disarmament Agency.

Issues discussed by this interagency committee, aside from the identification of research on rDNA molecules, have included: patent policies; the applicability of international conventions in restricting the use of rDNA techniques for the manufacturing of biological warfare agents; the need for special research facilities to conduct "high risk" and "risk assessment" rDNA research; the applicability of existing law to the regulation of commercial efforts to exploit the rDNA technique, consideration of Congressionally proposed legislation; and procedures for commercial production using rDNA techniques. Since 1980, no meetings have been announced, and the committee has no plans or projects underway to develop regulatory or policy initiatives or proposals affecting rDNA technology.

Regardless of the lack of action on the part of the interagency committee and in addition to tort law and workers' compensation, there are current federal statutes which could conceivably be implemented for regulating genetic technology and the resulting products:

- Federal Food, Drug, and Cosmetic Act (21 U.S.C. §301 et seq.)
- Occupational Safety and Health Act (29 U.S.C. §651 et seq.)
- Toxic Substances Control Act (15 U.S.C. §2601 et seq.)
- Marine Protection, Research, and Sanctuaries Act (33 U.S.C. §1401 et seq.)

- Federal Water Pollution Control Act, as amended by the Clean Water Act of 1977 (33 U.S.C. §1251 et seq.)
- The Clean Air Act (42 U.S.C. §7401 et seq.)
- Hazardous Materials Transportation Act (49 U.S.C. §1801 et seq.)
- Solid Waste Disposal Act, as amended by the Resource Conservation and Recovery Act of 1976 (42 U.S.C. §6901 et seq.)
- Public Health Service Act (42 U.S.C. §201 et seq.)
- Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. §136 et seq.)

1. Food and Drug Administration (FDA): The FDA is divided into four bureaus, roughly corresponding to the areas of jurisdiction provided in the Food, Drug, and Cosmetic Act. Pursuant to their respective regulatory powers, each bureau is responsible for the certification of a group of products as safe and effective.

The following FDA bureaus will be or are currently involved in regulating the existing or potential products of rDNA technology:

- National Center for Drugs and Biologics: human insulin, human growth hormones, thymosin, and other peptide hormones; endorphins, if they show therapeutic potential: DNA itself; interferon, vaccines (such as hepatitis B and influenza), and blood products like urokinase, serum albumin, and clotting factors.

- o Bureau of Veterinary Medicine: bovine growth hormone and interferon.
- o Bureau of Foods: certain enzymes and food additives used in food processing.
- o Bureau of Medical Devices and Radiological Health: monoclonal antibodies, in vitro diagnostic tests (e.g., testing components such as glucose oxidase) and specific tests for thalassemia.

Recently, the FDA has shifted from individual bureau policy development to the development of a coordinated regulation policy. The FDA has established an rDNA coordinating committee to decide on such procedures as how data requirements will be streamlined for biotechnology products whose active ingredients are identical to ones already approved and on the market.

FDA policy, proposed by the coordinating committee, was approved and issued in response to applications seeking approval of rDNA derived substances. All pharmaceutical products manufactured using rDNA technology will require new drug applications regardless of whether or not the products are identical or similar to naturally occurring substances or to previously approved products manufactured in a conventional way. The amount of data that must be contained in the required applications will depend on a variety of factors, including the proposed use of the product, how long the product is to be administered, previous clinical experience and the applicant's previous experience with products created by use of this new technology.

The National Center for Drugs and Biologics was formed in May 1982 by merging the two previously separate bureaus. The objective of FDA was to facilitate the creation of an improved science base for the drug review process and increase its ability to assure the safety and efficacy of drugs and biological products. As products related to biotechnology are reviewed by the National Center for Drugs and Biologics, a positive trend is emerging: industry and federal regulators are interacting more frequently and are sharing scientific expertise to address, more rapidly, questions related to product safety and efficacy.

Based on the demonstrated identity of molecular structure of natural and rDNA made products, it is likely that pharmaceuticals will qualify for the FDA's "fast track" New Drug Application review, although each product will be reviewed "case by case". The fast track at FDA involves more intense and frequent scrutiny prior to the review process for drugs that are "new" (not merely extant products manufactured by new technology), with the aim of expediting the approval and marketing of drugs that are not presently available and provide pharmaceuticals for new indications.

The regulation of veterinary products derived from biotechnology under the Bureau of Veterinary Medicine is analogous to regulations of products for human use. However, if the animals are food-producing animals, the drugs must also be shown to be safe for human consumption and must not leave unsafe residues in the animal-derived food for human consumption.

The division of regulatory powers between the FDA and the Department of Agriculture (e.g., Animal and Plant Health Inspection Service, APHIS) is not entirely clear in regard to the development of new plant strains, animal biologics, and drugs developed through modern genetics. For example, the FDA's Bureau of Foods would become involved it crown gall tumor viral genes were used to transform plants and code for nitrogen fixation. This Bureau would determine whether the tumor genes also produced a toxin of any kind that would be present in the edible tissues of the plant. APHIS would be involved only if such a nitrogen-fixing plant should become a carrier of a plant disease.

Products that may be manufactured using rDNA techniques include food additives. The Bureau of Foods requires pre-market review of all food additives which are defined as substances that, through their intended use, can become a component of food or otherwise affect the characteristics of food. The Delaney Clause prohibits the use of any food additive that has been shown to induce cancer when ingested in man or animal.

The Bureau of Medical Devices reviews products defined as any health care product that does not achieve its purpose by chemical action in or on the body or by being metabolized. Diagnostic kits are classified as medical devices and might include products of genetic technology. They would require approval for safety and effectiveness before they could be marketed.

Approvals for the diagnostic kits using hybridoma technology have been issued since May 1981. Many applications for the approval of monoclonal-based diagnostic kits have gone through the regulatory route known as the 510 (K) process. Most biotechnology firms developing hybridoma test kits hope to place them on the market by going through the 510 (K) process. However, Abbott Laboratories in North Chicago, Illinois, recently won clearance through another FDA channel, premarket approval (PMA). Abbott already had approval for two carcinoembryonic antigen (CEA) kits using conventional antibody technology. In July 1981, Abbott filed a supplemental PMA because it wanted to make the kits using monoclonal technology. Both kits were approved in March. Because the kits were classified as class III devices, the strictest of the classes in which FDA places medical devices, Abbott had to use the lengthier PMA regulatory channel. Abbott is the first company, then, to have gained approval on a class III device using monoclonals.

2. The Occupational Safety and Health Administration (OSHA):

OSHA has addressed potential hazards to workers related to commercial recombinant DNA applications. In 1980, OSHA communicated to the NIH RAC that OSHA planned to regulate rDNA operations under its current authority. Thus, OSHA retained the right to determine, in the course of an inspection, whether the workplace is in compliance with the NIH Guidelines. Ostensibly,

this includes the right to take action against those employers who are found to be out of compliance. However, to date OSHA has taken no further action.

3. Center for Disease Control (CDC): CDC has been indirectly involved in the development of the NIH Guidelines through its list of pathogenic agents, issued by the Center for Infectious Diseases, which classifies the agents according to their toxicity. The NIH RAC relied heavily on this list (and continues to do so) in its assignment of various rDNA experiments to various physical and biological containment levels and in its designation of experiments that require the explicit approval of the NIH. The more dangerous the microorganism (according to the CDC classifications), the higher the physical and biological containment levels assigned by the RAC.

The CDC has also issued draft "Biosafety Guidelines for Microbiological and Biomedical Laboratories." As stated in the introduction of these Guidelines, "The primary purpose of these proposed CDC Biosafety Guidelines is to provide recommendations for the safe handling of microorganisms that are known to cause or may cause human disease." The minimal recommendations of the CDC Biosafety Guidelines are applicable to training, clinical, diagnostic, research, and other laboratory activities in which viable microorganisms or clinical materials that are known to contain or may contain agents infectious for humans are handled

or worked with. In addition, the CDC (as well as the Department of Transportation) has the authority to regulate the interstate shipment of etiologic agents.

4. National Institute of Occupational Safety and Health (NIOSH): NIOSH is one of the seven operating divisions of CDC. Part of the NIOSH program, addressing potential hazards to workers related to commercial biotechnology, included on-site surveys of companies moving into scale-up or pilot production phases of operation. Initial walk-through surveys were conducted at rDNA facilities located at Eli Lilly, Genetech, Burns-Biotec and Cetus to assess the control measures and reduce human exposures. Information ob- tained from this program could be used to develop methods to minimize identifiable risks to workers and to advance recommenda- tions to specific companies. Topics that will be covered by the program include:

- o Physical containment design (e.g., fermenters)
- o Engineering controls (e.g., ventilation, exhaust gas filtration)
- o Work practices (e.g., operational protocols, clothing, and hygiene requirements)
- o Validation procedures (e.g., sterilization and fermenter containment)
- o Emergency procedures (e.g., spills)
- o Environmental monitoring

- o Medical surveillance (e.g., serum samples for serologic changes; checks for possible physiologic changes related to product exposure)
- o Worker education

"Medical Surveillance of Biochemical Workers," a Report on the CDC/NIOSH Ad Hoc Working Group on Medical Surveillance for Industry Applications of Biotechnology was published in the Recombinant DNA Bulletin, Vol. 5, No. 3, September, 1982.

5. Environmental Protection Agency (EPA): EPA has established an in-house group to evaluate existing legal and regulatory mechanisms available to the agency regarding the applied genetics industry. Exploratory efforts have also been initiated to evaluate beneficial uses of the products of this industry in reducing existing hazards to the environment. EPA has further conducted seminars dealing with containment levels and water decontamination. In 1982, EPA's Office of Exploratory Research issued a report entitled "Industrial Use of Applied Genetics and Organisms." The report makes specific recommendations to the EPA:

- o Any environmental risks arising from industrial uses of applied genetics are speculative. At this time, there exists no compelling reason for the EPA to establish regulations in this area.
- o Should environmental hazards emerge in the future, it is probable that they can be handled within the existing

regulatory framework (most notably Toxic Substances Control Act).

- o The EPA should continue to take an active role in promoting applied research and development of biological waste management processes and techniques, with particular emphasis on acquiring a better understanding of the biology of relevant systems rather than on process design and engineering.
- o The EPA should sponsor further investigation into the generation, dispersal, and control of biological aerosols.
- o The EPA should endeavor to monitor commercial and scientific developments in the field of applied genetics with the aim of identifying both imminent environmental hazards and areas where this technology might be applied to pollution control problems.

Response to these recommendations has yet to be evaluated. EPA, however, continues to be very actively engaged in developing its regulatory response to advances made possible through the use of rDNA technology. Current EPA activities include:

1. Seminar series with the American Association for the Advancement of Science on the effect of rDNA on the environment.
2. Testimony before Congressman Gore regarding jurisdiction under TOSCA to regulate deliberate release.

3. Sponsorship of risk assessment studies such as the Carnegie - Mellon study on potential release of rDNA organisms from large scale uses.

E. Conclusion

The federal regulatory environment currently monitors, through several agencies, developments in rDNA research. While improvements could be made in coordinating activities, it is recognized that the experimentation and production work utilizing recombinant DNA organisms has proven safe.

Part III. THE SOCIAL AND ETHICAL ISSUES OF HUMAN GENETIC ENGINEERING

Since the ability to manipulate genetic material was first recognized, scientists, religious leaders and the public have debated the potential social and ethical consequences of rDNA. Concern regarding the first stages of rDNA technology, i.e., the development and production of drugs and biologics such as interferon and insulin, has been allayed for the most part. The problems encountered in producing safe medications through gene splicing have been shown to differ little from problems with conventionally developed products. However, gene therapy and gene surgery in humans, the anticipated next phases of rDNA, will continue to generate questions as to the effect of this technology on our society.

A. Religious and Ethical Views on Genetic Experiments in Humans

Human beings have been creating new life forms since the cultivation of new characteristics in plants and breeding of new traits in animals was learned. The difference with rDNA technology is that it "breaches species barriers" by combining DNA from different organisms and does so by scientific design--not chance. The ethical question here is whether there is something intrinsically wrong with intentionally crossing species lines.

Among religious leaders, genetic engineering is often described as "playing God." For some, it expresses the view that

human beings are on the threshold of understanding how the fundamental machinery of life works. This expression, in their view, is not criticism but a recognition of the potential power of genetic engineering.

The phrase "playing God" can also reflect the view that scientists are abusing their knowledge by intefering with nature and altering existing organisms by adding a new characteristic. This is sometimes expressed as an "arrogant interference with nature."

In the report from the President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavorial Research released in November, 1982, none of the religious scholars reporting to the Commission, "suggested that either natural reason or revelation imply that gene splicing technology is unnatural in this prescriptive sense." All emphasized that human beings have the duty to use their knowledge in improving the human condition. According to these religious scholars, not to explore the potentials of genetic engineering would be unethical.

On the other hand, other clergy have adopted a resolution urging scientists to cease experimentation to alter human heredity. These religious leaders were not addressing the issue of altering genes to treat a specific congenital ailment, such as sickle-cell anemia. This type of gene therapy alters somatic cells only. Rather, the statement adopted by this group of religious leaders specifically objected to the alteration of

reproduction and "... getting into an area of creation that is the province of God." Such changes could be effected by direct manipulation of genetic material of zygotes or by indirect alteration of reproductive cells in adults during treatment of other inherited disorders.

In actuality, the positions of these two groups are not as divergent as appears at first glance. Both groups support the concept of gene splicing as "therapy" for treatment of congenital diseases. The disagreement occurs on the issue of altering reproductive cells for the prevention of inherited disorders. The resolution most recently adopted by a group of religious leaders condemns any experimentation that could alter traits inherited by future generations. The statement from the President's Commission reflects a balanced view of the potential benefits of such rDNA procedures with the voiced concern of potential problems and the recommendation for close scrutiny of research in this area.

B. Concerns About Social and Biological Consequences

There are some who object to genetic engineering because it interferes with the "wisdom of evolution." They view species lines as naturally protective barriers that should not be crossed because of potential serious consequences. Others are concerned about the loss of "heterozygote advantage", the protection that varied characteristics offer for survival of a species. While long-term effects are the most difficult to anticipate, these

evolutionary concerns should not be dismissed lightly and such potential impacts should be studied.

Another issue raised by genetic engineering is the possible societal obligations that could be incurred. As biotechnology advances and the ability to reduce genetic defects develops, the question of what society owes to children and future generations will need to be addressed. The burden that genetic diseases imposes on its victims and on societal resources may cause genetic treatments to be mandated. There is already a history of involuntary blood transfusions ordered by the courts when physicians conclude this necessary to prevent serious harm to the fetus.

Fi ally, the manipulation of genes that play an important role in regulating human growth processes or contribute to personality or intelligence could have an impact on individual's psyches. Genetic engineering might possibly provide quicker, more elective and easier means of making important psychological improvements in people suffering from mental illness. However, there is also the possibility that improving human traits through genetic engineering could lead to change in human nature. In this way, rDNA technology may help solve the controversy of nature versus nurture, but contribute unwittingly to a permanent change in the nature of humans.

C. Conclusion

Indirect manipulation of the human gene pool has been occurring for quite some time through the process of "natural selection." Compensating for myopia with eyeglasses and the attendant survival of the genes responsible for this condition has not had a deleterious effect on the human population. Likewise, it is known that certain therapeutic measures indirectly affect the human gene pool, e.g. treatment of diabetes, hemophilia and certain types of congenital heart disease, allowing the afflicted person to reproduce. The point is that indirect manipulation of the gene pool currently exists and can be medically beneficial depending upon the environment in which the genetic changes occur.

Direct manipulation of the gene pool (gene therapy) is a therapeutic development evolving from our knowledge and understanding of disease mechanisms. Genetic engineering is not a radical departure from previous methods of medical intervention. Altering or compensating for the phenotypic expression of diseased somatic cells is the manner in which medicine has proceeded from its inception.

A clear distinction in policies concerning genetic engineering should be made when discussing manipulation of the human germ cell. Unlike somatic cell gene therapy, the use of genetic engineering technology to alter reproductive cells would constitute a definitive departure from previous therapies

because future generations would be affected. These distinctive uses of rDNA need to be clearly defined when discussing the social and ethical considerations of this technology.

Part IV. BALANCING BENEFITS VERSUS CONCERNS

In order to place in perspective the social and ethical questions which have been raised concerning the results which may be possible through use of biotechnology, more specifically rDNA technology, by the pharmaceutical industry, one must not only analyze these concerns but also evaluate them against the benefits which are possible. The preceding chapters have discussed what biotechnology is, how it has been regulated, the types of concerns which have been raised regarding its potential misuse in human experimentation, and only partially reviewed the benefits which are possible through use of this new technology. One must not diminish the importance of the latter point since the potential benefits are truly substantial.

The pharmaceutical industry has and will continue to use rDNA technology in a responsible manner. Most importantly, rDNA technology will create therapeutic and diagnostic products which are found in nature but which otherwise could not be produced economically for commercial use. Examples of this include human insulin which is now being marketed in the United States and several other countries, human growth hormone which is under clinical investigation and several forms of interferon which are also under clinical investigation. Also, through use of this new technology, it is possible to improve production efficiences for existing products. This in the long run will result in better control over escalating costs in this industry. Without rDNA technology, the benefits and potential benefits of

these products and many more to come would not have occurred. The positive benefits to mankind are, therefore, truly substantial with respect to this applicaton of rDNA technology.

Up to this point and in the immediate foreseeable future, the above uses of rDNA technology comprise the extent of the research commitment in which the PMA member firms are engaged. No member firm has performed interventions genetically in humans to treat known genetic defects nor has any member firm performed in humans other genetic investigations such as genetic engineering experiments. It is in these latter two types of investigations that almost all of the social and ethical concerns about the use of rDNA technology focuses.

Given the benefits possible and the responsible manner in which the pharmaceutical industry has conducted itself, the following is PMA's position on biotechnology issues.

Guidelines or Other Forms of Regulatory Control

With respect to external controls, the PMA supports continuation of the National Institutes of Health Recombinant DNA Advisory Committee (RAC). RAC has developed guidelines which are applicable to all federally funded research. More importantly, all member firms of PMA have voluntarily agreed to comply with the spirit and intent of these guidelines. Thus, rDNA investigations in terms of safety to the environment and to the workers are adequately covered. This means that the NIH through RAC can continue to set the

guidelines for experimentation with rDNA technology and not become a regulatory agency. For the pharmaceutical industry, that role will be assumed by the FDA at an appropriate time in the development process.

There is no need to create a new federal agency to regulate "genetic engineering" experiments. Existing statutory authority provides adequate protection to responsibly set the ground rules for the use of rDNA technology. The NIH RAC has been recognized by Cabinet-level departments, regulatory agencies, and industry as being the central oversight and review organization for both the private and public sectors. Thus, at this time, there is no need to reactivate the Federal Interagency Committee on Recombinant DNA Research. However, should the role of the NIH RAC diminish, then we would recommend that the Interagency Committee be reactivated. Overall coordination of Federal rDNA activities saves money and prevents confusion over Federal policy in this area. Given the existing federal activity through the NIH RAC, we believe that control at the federal level is more than adequate. There is no need for additional state and local regulations.

In the event that a President's Commission on the Human Applications of Genetic Engineering is formed, the recommendations set forth above need not change. Such a Commission only adds another federal dimension to the review of

investigations in human beings utilizing rDNA technology. Since the power of the Commission is limited to conducting studies as opposed to regulating, the PMA should actively participate in the process of selecting members to such a Commission and in the establishment of its agenda.

Member firms of the PMA have limited their experimentation utilizing rDNA technology to creating therapeutic or diagnostic products which do not alter the human genome. However, there may be other organizations or non-PMA member firms which intend to intervene genetically in humans to treat genetic defects or to perform in humans other genetic investigations such as genetic engineering experiments. With respect to these latter two categories of investigations, the PMA supports the NIH RAC's proposal to monitor such research. This advisory committee should have borad-based representation in order to ensure proper consideration of the social and ethical issues which have been raised relative to these types of experiments. Ethical considerations are part of the deliberations of existing advisory committees when asked to review proposed protocols. "Non-scientific" representation on this advisory committee will not create a negative precedent given this factor and the unique nature of the issues being addressed. The advisory committee should establish guidelines for these types of investigations and also, at least initially, review all protocols relative to these investigations.

If, however, the RAC abandons review of rDNA research in humans, then the FDA should create a new advisory committee whose charter is to review INDS in this area. In the final analysis, the FDA will have to set the parameters for those investigations in which INDs will be required. An advisory committee whose sole task is to provide guidance in this area where science and social questions intertwine would be most helpful and lead to a better result than if the FDA did it alone.

Outside the pharmaceutical industry for which RAC and the FDA will set the regulatory guidelines, the major federal agency which will be involved in rDNA experiments is the Environmental Protection Agency. The EPA should and will set the guidelines for the widespread use of rDNA products intentionally released into the environment. This important role will be aided by the fact that it is a member of the Federal Interagency Committee on Recombinant DNA Research. It can put that membership to good use by learning from the FDA how it has handled questions regarding rDNA experiments.

Public Discussion and Understanding of the Benefits of rDNA Technology

Member firms of the PMA have invested and will continue to invest heavily in products developed through the use of rDNA technology. Because of public awareness that the pharmaceutical industry is using this new technology, the PMA as

spokesperson for the major pharmaceutical companies will be called upon to comment publicly about this new technology. The PMA should actively participate by testifying before Congress and setting forth in its testimony the recommendations made above. In addition, the PMA should develop and activate a low-key but deliberate and steady information effort to inform and educate policy-makers to understand and represent the pharmaceutical industry's position. In addition, it should be prepared, as circumstances warrant, to do the same with interested media, religious and public interest groups.

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April 25, 1985

Dr. William S. Gartland, Jr.
Director, Office of Recombinant DNA Activities
National Institute of Health
Building 31, Room 3B10
Bethesda, Maryland 20205

Dear Dr. Gartland:

Subject: FR Doc 85 - 7064
Recombinant DNA: Notice of Meeting and Proposed Actions Udner Guidelines for Research

We are writing to comment on the proposed Amendment to Part III-A-1 of the Guidelines for Research Involving Recombinant DNA Molecules. Such amendment would establish a procedure for handling research proposals which are submitted concurrently to RAC and another Federal agency for review and approval. The amendment would permit NIH the option of deferring review and approval to the other Federal agency.

At the present time, the Office of Recombinant DNA Activities and the NIH Recombinant DNA Advisory Committee represent the only formal government body with the expertise and large base of experience to evaluate research proposals. Genentech agrees that dual review of proposals would seem unnecessary; however, concern also exists that at this time, various Federal agencies presented with rDNA proposals may not have all of the necessary expertise and pertinent experience in-house to thoroughly review the proposals. Since the function of such review includes determination that the experiment will not adversely affect man and/or the environment, it is especially important that the appropriate experts evaluate the information. To receive proper evaluation, and until such time that Federal agencies have in place a biotechnology review policy such as that proposed by the Office of Science Technology Policy, Genentech suggests a revision of Dr. Talbot's amendment to read as follows:

> "If experiments in this category are submitted for review to another Federal agency, the submitter shall notify ORDA and may provide information regarding the necessity of dual review; ORDA may then determine if dual review is necessary based upon whether or not the reviews serve the same purpose and whether the other Federal agency has the appropriate expertise and experience to thoroughly review the proposals in a timely manner, and . . ."

Genentech believes that RAC should enjoy the privilege of exercising an option to defer review, and it is our belief that this revision to the amendment still permits RAC the desired option, while continuing to provide constructive advice to those conducting research and protection of the public and environment.

We appreciate the opportunity to comment on the proposed amendment and would be pleased to answer any questions you might have regarding our suggested change.

Sincerely,

Carol Lax Gronbeck, Ph.D.
Biosafety Officer

cc: M. Ross
S. Ronspies
J. Swartz
E. Gazdeck

CLG:jaf

Monsanto

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WILL D. CARPENTER
General Manager, Technology

April 26, 1985

Director
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Building 31
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Bethesda, Maryland 20205

Dear Sir:

Monsanto Company is pleased to respond to the National Institutes of Health proposed action regarding Proposed Points to Consider for Environmental Testing of Microorganisms , which appeared in the Federal Register of April 25, 1985, 50 FR 12456-12458. Monsanto has publicly disclosed its position on the regulation of products developed through the emerging science of biotechnology. We believe an adequate federal, legislative, and regulatory framework is in place. New agencies and laws are unnecessary, so long as present agencies exercise existing authority consistently, cooperatively, and in a scientifically appropriate manner. Such a regulatory approach promotes the dual objectives of protecting man and the environment, and encouraging growth of the new industry.

We strongly recommend that the NIH Recombinant DNA Advisory Committee coordinate and harmonize its data requirements for environmental testing of microorganisms containing recombinant DNA with other Federal Agencies that have regulatory jurisdiction over such environmental testing. An applicant seeking approval for such environmental testing, whether they be an academician applying to the NIH-RAC, or a company applying to the Environmental Protection Agency, to the U. S. Department of Agriculture or the Food and Drug Administration should face the same data requirements to demonstrate the safety of the proposed environmental test. Procedures designed to evaluate field test protocols must be consistent whether the protocol is submitted by a researcher from a commercial concern, or from the non-profit/university sector.

We believe that reviews for the products of biotechnology should be made on a case by case basis.

We strongly recommend that reviews for such proposed tests and products be made in such a manner so as to protect the proprietary nature of submitted information.

The NIH-RAC must coordinate with other federal agencies, as listed in the Office of Science Technology Policy Federal Register Notice 49, FR 50855 regulatory matrix for biotechnology, to insure that no applicant is faced with a "double jeopardy" situation of seeking approval from more than one regulatory or oversight group for a proposed environmental test of a microorganism containing recombinant DNA. Multiple overlapping or sequential federal agency reviews must be obviated.

Specific concerns and questions which Monsanto has regarding the March 28th Federal Register notice are as follows:

1. Part II, Section A, subpart 2

   Clarification and better definition of "capacity for genetic transfer" is requested. As stated, it is unclear whether information regarding genetic stability and/or genetic exchange is being requested.

2. Part II, Section B, subpart 1a

   The inferred requirement for the sequence of the DNA used as the source of introduced DNA is not felt to be necessary. The source and function of the introduced DNA are considered to be reasonable requirements.

3. Part II, Section B, subparts 2a-c

   Requests for information on the modes of construction and introduction of vectors are not felt to be routinely justifiable, based on the potentially highly proprietary nature of such information. In those limited instances where such information was considered to be critical based on a case-by-case review, provisions should exist to protect the highly proprietary nature of that information.

4. Part II, Section B, subpart 3

   The term "genetic stability" should be defined more precisely.

5. Part III, Section A, B, and C

   Much of the requested information would not readily be available, even on a long-term research basis. The proposed requirements are too broad, too general and too all encompassing to be realistically met. Reasonable limits need to be defined and definitions for terms such as "shifts in important ecological characteristics" and "exchange of genetic information between the modified organism and other organisms in nature need to be clarified.

6. Part IV, Section A (Pre-Field Trial Considerations)

Better definition of the term "microcosm" is requested. It may well be that greenhouse or growth chamber experiments indeed represent microcosms of microbes, soil, plants, etc. Better definition of (non-target) "mobile organisms" is requested.

7. Part IV, Section A, subpart 3

Establishment of better defined, reasonable limits for estimating "dissemination by wind, water, soil, mobile organisms and other limits" is requested.

8. Part IV, Section D

The proposed requirement for monitoring "non-modified" microorganisms at the site of the trial is not felt to be either reasonable or technically feasible unless "non-modified" organisms are defined as spontaneous or induced genetic mutants which have not been genetically engineered. Such non-genetically engineered, but genetically marked strains could be tracked in the field by the use of appropriate selective microbiological media as has been amply shown in the Rhizobium and Pseudomonas literature, with the use of e.g., rifampicin resistant and naldixic acid resistant mutants.

Monsanto appreciates the opportunity to provide our comments to the NIH-RAC ORDA Working Group on Release of Organisms to the Environment for consideration in its final draft of Points to Consider in The Deliberate Release of Microorganisms to the Environment.

Sincerely,

Will Carpenter

Will D. Carpenter

INDEX OF LETTERS AND DOCUMENTS

Name	Page
Baier, Edward J.	40
Berg, Paul	42
Berns, Kenneth	266
Brill, Winston J.	271
Brown, Joseph P.	274
Carpenter, Will D.	637
Castro, C. Elizabeth	277
Cavalieri, L. F.	43
Gliessman, Stephen R.	301
Goldberg, Terri.	34
	593
Gore, Albert Jr.	351
Gronbeck, Carol Lax.	635
Gustafson, Donald P.	269
Halvorson, Harlyn O.	266
Horowitz, Sheldon.	30
Jameson, Alice K.	276
Jennings, John	595
Johnson, Irving S.	282
Jones, Mary Ellen.	288
Juengst, Eric T.	37
Kass, Leon R.	63
Lee, James O., Jr.	41
Levine, Myron M.	355
McDermott, T. J.	44
McKinney, Robert W.	32
Miller, Henry I.	46
	357
Pramer, David.	266
Pimentel, David.	296
Price, Harvey.	280
Rifkin, Jeremy	45
Riley, Monica.	266
Rogers, Edward Lee	291
	352
Sherman, John F.	272
Singer, Maxine	29
Spurrier, Earl C.	286
Suslow, Trevor	290
Vidaver, Anne K.	278

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